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# PHYTOPATHOLOGY

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## CERCOSPORA LEAF SPOT OF EGGPLANT

COLIN G. WELLES

WITH TWO FIGURES IN THE TEXT

A serious spotting of the leaves of eggplant (*Solanum melongena* Linn.) has been observed recently in the Experiment Station, at the College of Agriculture, Los Baños, Laguna Province, Philippine Islands. Leaf spotting of eggplant is quite commonly caused by the fungus, *Phyllosticta hortorum* Speg. but the leaf spot about to be discussed is not similar to it in any respect. This paper presents the results of studies made on the disease with special emphasis on the causal organism and control.

### THE DISEASE

*Symptoms.* The spots occur in abundance on the leaves, the old leaves being most seriously attacked. The young lesions appear as chlorotic spots, irregular in shape and frequently fusing, giving the leaf a yellow, mottled appearance. The irregular shape is due to the limiting of the lesions by the veins of the leaf. The young lesions develop on leaves of all ages but chiefly on the lower, old leaves. They first appear on the upper surface of the leaf, later extending through the tissues and causing a spot on the under surface as well. When they become older the central portions of the lesions dry out and turn grayish-brown or dirt color due to the death of the cells, and exhibit concentric rings. With age these dry areas may become 6 to 8 mm. in the largest dimension. The dry portions develop first at or near the margins of leaves and spread inward frequently as much as 20 mm. These lesions are always surrounded by a band of yellow tissue. In very advanced stages of spotting, the dead tissues fall out leaving a shot-hole condition.

The fruit of many plants has been carefully examined but no lesions have been found which can be traced to the Cercospora.

No disease of eggplant has been described as far as can be ascertained, which approaches the appearance caused by this fungus more closely than the leaf spot due to *Phyllosticta hortorum*. However, the lesions of this disease are larger in size and lighter in color than the Cercospora leaf spot under discussion. Futhermore, the pycnidia of the Phyllosticta are very pronounced whereas the Cercospora lesion is evenly colored and unbroken by any dark sporiferous bodies.

*Varietal attack.* The Cercospora is confined to eggplant so far as is now known. Within this host species, however, observations point to varieties showing differences in susceptibility to this parasite. The native Philippine eggplant which has long, black fruit, turning yellow at maturity, is very seriously parasitized while a Siamese variety having small, round, yellow fruit is but slightly affected The native variety was seriously attacked from the end of the dry season but the Siamese variety showed no signs of disease until nearly four weeks later.

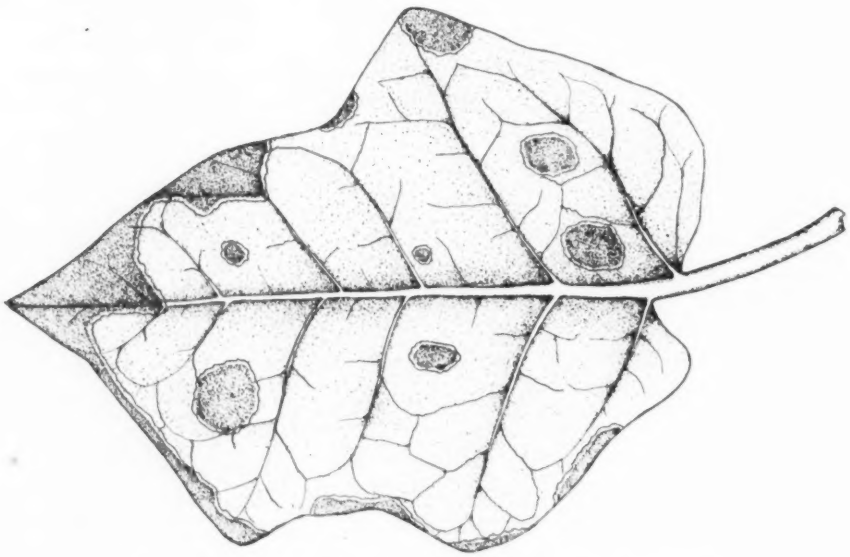

Fig. 1. Leaf spot of eggplant caused by Cercospora melongenae.
Drawing by C. C. Nacion.

*Economic importance.* Plants grown in the Experiment Station are severely attacked by the Cercospora. On fully matured leaves the photosynthetic area of the leaf is reduced more than 75 per cent. On young leaves which are still developing, the lesions are confined to the margins with a reduction of photosynthetic tissue often reaching 30 per cent. All plants in the field are affected by the fungus with from 50 to 100 per cent. of the leaves attacked.

*Distribution.* The disease has been observed only on plants in the Experiment Station. A survey of the market gardens of the Philippine Islands will be necessary before the extent and seriousness of the disease in other places can be ascertained.

## THE CAUSAL ORGANISM

*Morphology.* The organism which causes the leaf spot of eggplant belongs to the genus Cercospora. The fruiting bodies are borne in the grayish-brown center of the lesions, mainly on the lower surface of the leaf. The conidiophores are of the common Cercospora type, arising in bunches from the stomatal openings. They are rather short, simple, light brown, and erect. They measure from 30 to 60 microns long and 4 to 6 microns wide and are 2 to 4 septate. The conidia are linear, slightly larger at the basal end, tapering gradually to a blunt point and hyaline. The average conidium measures 74.85 by 7.14 microns, the extremes being 38 to 119 microns long and 4.7 to 8.3 microns wide. They are 3 to 12 septate.

*Cultural characters.* Germination took place in 12 hours in our trials. The conidia germinated by slender germ tubes, one, two, or three generally arising from each conidium from as many cells. The fungus may be isolated by the single spore method in potato agar or by plating diseased tissue directly on corn meal. The mycelium development is rapid. On potato-glucose agar the mycelium when young is white and fluffy and grows close to the medium, later becoming a very thick, closely matted mass with a grayish-green center encircled by a band of white mycelium. The cultures have been kept for five weeks but no fruiting structures have developed.

Since the organism is apparently a new species the following name and description is submitted:

**Cercospora melongenae** n. sp. Spots amphigenous, indefinite, grayish-brown, 6 to 8 mm. in largest dimension, concentric rings. Conidiophores mostly hypophylous, short, light brown, fasciculate, 30–60 x 4–6 microns, 2–4 septate. Conidia filiform-obclavate, straight or curved, 38–119 x 4–8 microns, averaging about 75 x 7 microns, 3–12 septate, hyaline.

Found on eggplant, *Solanum melongena* Linn., in Los Baños, Laguna Province, Philippine Islands.

## CONTROL

Several experiments have shown that spraying with Bordeaux mixture every two weeks has sufficed to hold in check the development of the leaf spotting of the eggplant. However, comparison of such sprayed plants with the unsprayed controls has shown no important difference so far as relates to the setting or development of the fruits. On the other hand, the spraying does increase the longevity of the leaves and

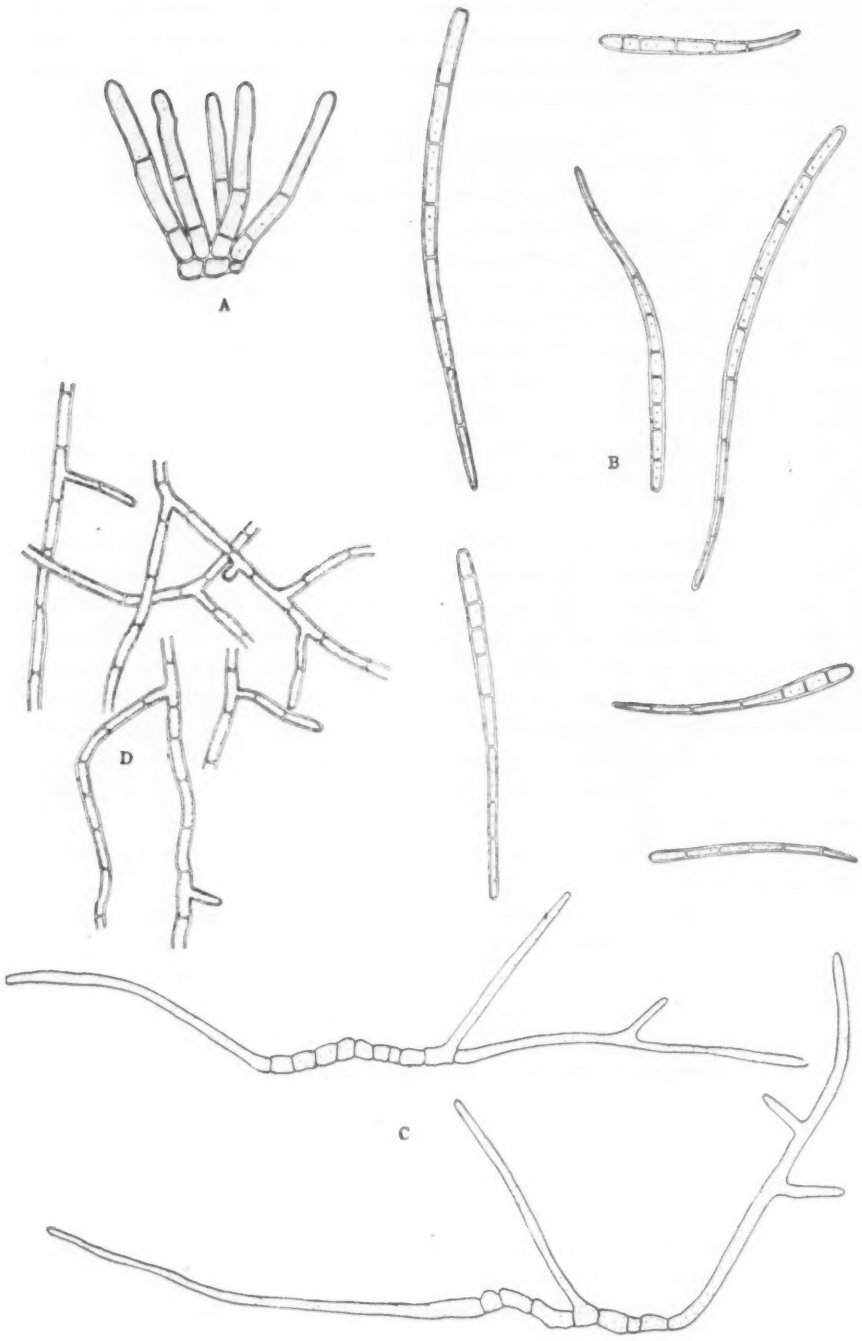


Fig. 2. *Cercospora melongenae:* A, group of conidiophores. B, conidia. C, germinating conidia from potato-glucose agar plates. D, mycelium from pure culture, on potato-glucose agar. Camera lucida drawing by E. Roldan and C. C. Nacion. × 460

the general vitality of the plants. From these results it seems that spraying for such control purposes is not commercially justified for mature plants unless the disease threatens to be so unusually severe as to cause serious defoliation. Should this occur, especially with young foliage or young plants, spraying may be relied upon as a control measure.

SUMMARY

1. A previously unreported Cercospora leaf spot of eggplant has been found in the Experiment Station for the College of Agriculture, Los Baños, Philippine Islands.

2. The lesions look somewhat similar to those caused by *Phyllosticta hortorum* Speg. The general appearance of the lesion is the only point of likeness however.

3. It attacks all local varieties but is most severe on the native Philippine eggplant. It is less parasitic on a Siamese variety with round, yellow fruit.

4. It may reduce photosynthetic tissue as much as 75 per cent.

5. The causal organism is described as a new species under the name *Cercospora melongenae*.

6. The leaf spotting is easily reduced by spraying with Bordeaux mixture. Since this has not materially increased the yield with mature plants it may not be commercially profitable unless the attack appears with unusual earliness or severity.

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# THE TAKE-ALL DISEASE OF CEREALS AND GRASSES

R. S. KIRBY[1]

WITH PLATES II TO IV AND THREE FIGURES IN THE TEXT

The take-all disease of wheat was discovered for the first time in the United States in July 1920 (13). Since that time extensive investigations have been made at Cornell University and the results are presented in this paper.

The name take-all has been generally applied, in Australia, England, and other English-speaking countries, to a disease produced by *Ophiobolus cariceti* (B. & Br.) Sacc. While it has been applied to forms of foot rot which are due to other causes, it seems desirable to restrict the name to the disease caused by this species of Ophiobolus.

## GEOGRAPHICAL RANGE OF THE DISEASE

An extensive historical and bibliographic treatment of various foot rots of wheat has been published by Stevens (26). Since many writers have only recently associated Ophiobolus specifically with the foot rot which it causes, many statements in the older literature are of little value in determining the host range of the fungus. The causal organism has been known since 1861 (1), when it was described from specimens collected at Batheaston, England, on *Aira caespitosa*. In 1875 it was described as occurring on *Agropyron sp.* and *Cynodon sp.* in Italy (24), and in 1878 it was noted on wheat in France (20). Take-all of wheat was found in England in 1884 (10), and in 1890 it was described in France (22). McAlpine (16) found the disease widespread on wheat in South Australia in 1904, and he states that it has been known since 1852.

Take-all has been observed in France, England, Holland, Belgium, Portugal, Italy (19), Australia (16), New Zealand (31), Japan (29), Denmark, Russia (7), and the United States (13).

[1]Suggestions and help were received from Doctor H. E. Thomas during the earlier part of these investigations.

As herein defined, it was first recorded in the United States by Kirby and Thomas (13). However, since that report was published, the disease has been found in Oregon (11), Arkansas (11), Indiana,[1] and additional localities in New York State (12).

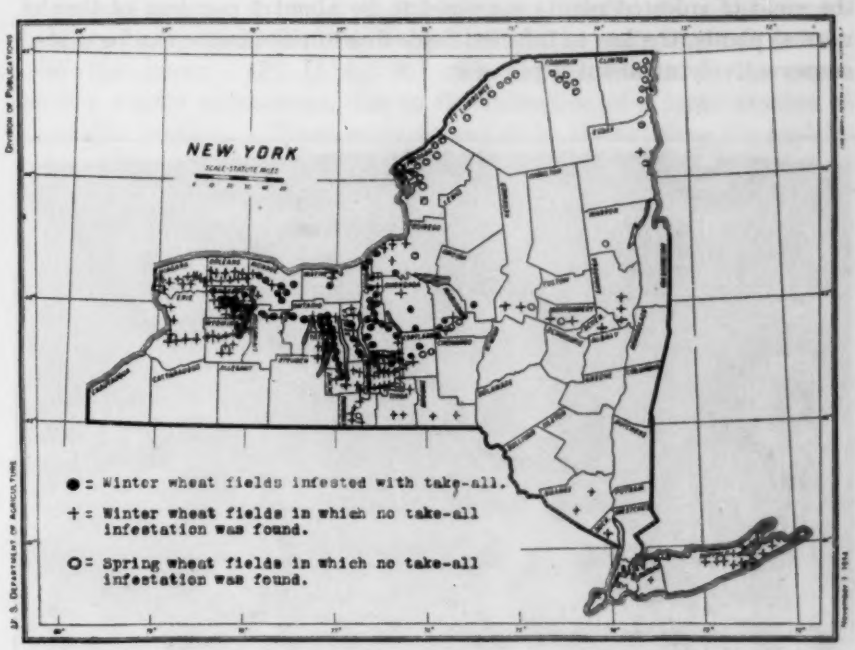
NEW YORK
SCALE-STATUTE MILES
● = Winter wheat fields infested with take-all.
+ = Winter wheat fields in which no take-all infestation was found.
○ = Spring wheat fields in which no take-all infestation was found.

FIG. 1. LOCATION OF ALL CEREAL FIELDS SURVEYED IN 1921 WITH LOCATION OF TAKE-ALL INFESTED FIELDS.

A survey was made in 1921, in cooperation with the office of the Plant Disease Survey of the United States Department of Agriculture, to determine the distribution of take-all in New York State. The survey extended over thirty-six counties and included all sections where an appreciable amount of spring or winter wheat was grown. The location of all take-all infested fields, as well as all other winter and spring wheat fields surveyed, is shown in figure 1.

Plants affected with take-all were found in 78 of the 224 winter wheat fields surveyed. All of the 78 infected fields were located in sixteen counties in the west central part of New York. This infected area seems to have definite boundaries and closely coincides with the principal winter-wheat-producing area of the State. Within this area, fields representing more than 60 per cent of the total acreage surveyed were found to be infected.

[1]Reported to the writer in a letter from Professor H. S. Jackson, dated July 2, 1921.

An accurate determination of the amount of damage caused by take-all was impossible because many of the plants were killed in the seedling stage. However, some estimate of the resulting damage may be made from the fact that an average of 2 per cent of the plants in the 78 infected fields were killed by the take-all fungus. Therefore, since the yield of infected plants appeared to be about 1 per cent of that of normal plants, the loss in infected fields due to this disease can be placed conservatively at about 2 per cent.

Fig. 2. A WINTER WHEAT FIELD AT SPENCERPORT, N. Y., AT HARVEST TIME SHOWING AN AREA IN WHICH PLANTS HAVE BEEN KILLED BY TAKE-ALL.

## SYMPTOMS

The writer has not had the opportunity to study the disease through all its early stages in the field. The plants in the diseased areas at flowering time are stunted and bleached yellowish in color. From one to many plants are found in the infected areas which may attain a diameter of 15 feet or more and are usually circular in shape. (Fig. 2.) The edges often overlap in badly infected fields, so that one may walk across such a field without leaving them. Sometimes the disease occurs for several feet along the drill rows. The individual plants in these areas are usually ashy white in color, and are dwarfed to a few inches in height. They are usually dead and there is seldom more than one head to a stool. This chlorotic condition of the entire plant suggests the

name "white heads," which is used by some writers to designate this disease. There is a dark brown or black zone at the base of the diseased culms which is due chiefly to dark fungous mycelium in the leaf sheaths and between the culm and the inner leaf sheath, where it occurs as a plate-like mass. This discoloration is confined to the first and second internodes and may extend from one-half to two inches above the ground.

When badly diseased plants are pulled up, the roots break at or very near the crown. (Pl. II, fig. A.) The larger roots near the crown have a woolly appearance, due to the formation of a large number of short fine rootlets. These roots appear to be thicker than the healthy ones and retain considerable amounts of soil which often gives them a characteristic clubbed appearance.

One of the characteristic symptoms is the marked reduction of the normal number of tillers, many of which die soon after their formation. In plate II, figure B is shown the contrast between a healthy plant and a typical diseased one.

There is no marked change in the symptoms from the time of flowering to harvest of the wheat. However, the diseased leaves and culms may become sooty on account of the growth of saprophytic species of Cladosporium and Mucor. (Pl. III, fig. A.) At harvest time this blackening of the heads is as characteristic as the white-head stage is at flowering time. After the death of diseased plants, or rarely before, the beaks of the perithecia of the pathogene may protrude through the outer leaf sheath of the plant. (Pl. III, fig. B.).

The symptoms of the one infected rye plant observed differed from those on wheat in that there was little or no stunting of the plant and only slight shriveling of the kernels. The mycelial plate was less pronounced, and fewer perithecia were developed.

The chlorosis and stunting was not conspicuous on *Agropyron repens* (L.) Beauv., but the base of the culms was darkened by hyphal strands which were scattered in and between the leaf sheaths instead of being aggregated in a definite mycelial plate. Perithecia, however, were generally not so numerous as on wheat.

### COMPARISON OF THE SYMPTOMS OF TAKE-ALL CAUSED BY OPHIOBOLUS CARICETI, WITH "FOOT ROT" OR SO-CALLED "TAKE-ALL" DISCOVERED IN MADISON COUNTY, ILLINOIS

The disease called foot rot by Stevens (26) has several times been referred to as "take-all" (11) or so-called take-all (17). It has the following symptoms in common with the take-all as it occurred in New York in 1920 and 1921: the stunting and death of plants in rather definite spots in wheat fields; the blackening of the lower culm usually the first internode.

The take-all in New York differs from the Illinois "so-called take-all" in the following respects: between the leaf sheath and the culm of infected plants, there nearly always occurs a definite plate of brown mycelial strands (Pl. III, fig. C.) Typical Ophiobolus perithecia containing ascospores were found on plants in every infected field. From infected plants cultures of *Ophiobolus cariceti*, *Fusarium graminum* Corda,[1] *F. avenaceum* (Fr.) Sacc.,[1] *F. diversisporum* Sherb.[1] and *Gibberella saubinetii* (Mont.) Sacc. were obtained, but *Helminthosporium sp.* never was isolated. Helminthosporium was often found associated with the plants in the later stages of the Illinois disease, which always lacked the perithecia and the typical plate mycelium of the take-all organism. The diseased plants in New York differ from those in Illinois also in that they exhibit hypoplastic instead of metaplastic tillering symptoms in the spring.

## IDENTITY AND DESCRIPTION OF THE FUNGUS

In an earlier paper (8), the causal fungus of take-all was shown to be *Ophiobolus cariceti* which is identical with the take-all fungus in Australia, Japan, England, France, and Italy.

The take-all fungus has been grown in pure culture on the following agar media: potato, lima bean, cornmeal, oatmeal, prune, nutrient, Czapek, and 0.2 per cent, 2 per cent, and 5 per cent dextrose. The growth of the fungus on these various media was of three general types, examples of which are described.

### GROWTH ON 0.2 PER CENT DEXTROSE AGAR

Growth 2.5 cm. in diameter after seven days; seldom reaching a diameter finally of more than 5 to 7 cm.

*Growth after seven Days*: The mycelium is submerged or wholly appressed. The individual fungous growth is hyaline, and is made up of feathery, rhizomorph-like strands resembling the feathery branching of a moss.

*Growth after thirty Days*: Appearance nearly the same as at end of seven days, except *deep neutral gray*[2] hyphal strands have appeared near the center. The rhizomorphic strands are appressed to the surface or submerged. The strands radiate toward the edge of the culture and do not unite nor run parallel. The cells of the rhizomorphs are from 16 to 45μ long and from 4 to 5.5μ in diameter, and contain from 10 to 25 guttulae. The cell walls are hyaline. Branches arise from near the tip of about every eighth to tenth cell, or about every 150 to 250μ. The cells of the branches are very much more variable in size and shape than those of the main rhizoids, ranging from knob-like cells with a diameter of 5μ, such as occur at the end of a few hyphae, to cylindrical cells resembling those of the main rhizomorphs. (Pl. IV, fig. A.)

[1]Determined by Doctor C. D. Sherbakoff.

[2] Ridgway color.

### GROWTH ON 2 PER CENT DEXTROSE AGAR

Growth 4 cm. in diameter at the end of seven days, covering the plate (9 cm.) in from four to five weeks

*Growth after seven Days*: The mycelium is hyaline, and is submerged or wholly appressed. It is much less characteristically rhizomorphic than in 0.2 per cent dextrose agar, but this character is evident. The individual strands are finer and more abundantly branched, and their margins are uneven and indistinct.

*Growth after thirty Days*: The surface of the fungous growth is of a deep neutral gray color. The mycelium is submerged or wholly appressed. The individual strands appear often distinctly frayed at the tip, forming a rather definite tuft which continues as lighter colored hyphae. Many ribbon-like rhizomorphic bands run irregularly over the surface of the medium. These bands are made up of from two to three (sometimes as many as six) strands of hyphae running parallel. They have fewer hyphal strands than are produced in cultures grown on potato agar. The cells of the individual strands are cylindric and have thick deep neutral gray walls. They are from 25 to 275μ long and from 4 to 6.5μ in diameter (averaging 196 × 5.5μ). The contents are hyaline and the cells contain from 0 to 40 guttulae. Branches arise alternately, usually one, rarely two, from the distal end of nearly every cell.

Another type of growth often occurs in which there are numerous individual strands, and occasional ribbon-like bands rarely consisting of more than two hyphal strands. The individual cells of this type are cylindric and often constricted at their septa; they have thick, deep neutral gray wall, hyaline contents and from 10 to 40 guttulae; they range from 15 to 200μ in length (averaging 30 × 5.5μ). Branches arise from the tip of every eighth to fourteenth cell.

The branches terminate in hyaline hyphae whose individual cells are comparatively thin-walled, very variable in shape and size, ranging from 15 to 60μ long and from 2 to 6μ in diameter. The cell contents are guttulate. Anastomosing was observed. (Pl. IV, fig. D.)

### GROWTH ON POTATO AGAR 5.4 PH

Growth 7 cm. in diameter at the end of seven days, covering the plate (9 cm.) in from eight to nine days.

*Growth after seven Days*: The mycelium is hyaline, and is submerged or wholly appressed. The individual culture is abundantly and distinctly flexuous; its margin is even but not marked by a definite line, appearing finely frayed.

*Growth after thirty Days*: The mycelium assumes a more pronounced aerial habit, rising to a height of 2 mm. and growing on the sides of the petri dish. The aerial growth causes the surface of the colony to appear as if covered with a silvery bloom. The surface of the medium is thickly covered with tortuous, mixed ribbon-like rhizomorphic bands of hyphae. The surface of the colony ranges in color from a neutral to a deep neutral gray. The rhizomorphic bands are made up of from 2 to 8 strands of parallel hyphae. The cells of the individual strands are cylindric and have thick dark neutral gray walls; they are 75 to 260μ long and from 4 to 6.5μ in diameter (averaging 184 × 5μ); the contents are hyaline and sparingly guttulate. Nearly every cell in the strands is branched at its tip. The cells of the hyphal strands closely resemble those of the plate mycelium on the host. The branches terminate in thin-walled hyaline hyphae, the individual cells of which contain from 10 to 30 guttulae. The cells are very variable in shape and size, ranging from 15 to 50μ long and from 2 to 6μ in diameter. Anastomosing was observed. (Pl. IV, figs. C. and D.)

PARASITISM OF THE TAKE-ALL FUNGUS

In an effort to prove the pathogenicity of the fungus, Delacroix (6), Mangin (18), and McAlpine (16) inoculated growing wheat with diseased straw or spore suspensions, obtaining typical symptoms of the disease in each case. The first inoculations with a pure culture of the fungus were made by Waters (30) in New Zealand on wheat seedlings grown from sterilized seed on sterilized soil in test tubes. Inoculated plants died in from twenty-eight to thirty-six days from the date of inoculation, while control plants were in good condition after fifty-eight days. Inoculated culms of wheat plants growing in pots did not become infected.

In the present investigation the relation of *O. cariceti* to take-all was determined by the established rules of proof. At harvest time the base of the culm of infected plants was overrun with species of Fusaria, molds and bacteria. Nevertheless, pure cultures were obtained by first immersing bits of the host having one or more perithecia, in a 1-2000 solution of mercuric chloride for from one to two minutes to kill as many bacteria as possible. The material was then placed under a binocular microscope where individual perithecia were separated from all bits of the host tissue. These were placed in a few drops of sterile water on a slide where they were crushed. This suspension of ascospores was usually diluted to the desired degree and then atomized with a pipette having a very small delivery orifice on the surface of the medium in a petri dish. By placing a large drop of the suspension on the surface of the medium, then spreading it thinly over the surface some ascospores could be removed without bacterial contamination.

Two-tenths of one per cent dextrose agar was used throughout the isolation work. This medium is transparent, retards bacterial growth, and the take-all fungus growing on it produces a characteristic growth distinguishable from that of contaminating fungi. Single spore cultures were then obtained by transferring to sterile media young colonies which were observed under the microscope to have arisen from one ascospore.

By the use of the preceding methods during November and December of 1920, seven isolations were obtained from single ascospores and seven more from groups of two or more ascospores. Since that time it has been possible to make isolations whenever ascospores were germinating. After obtaining the fungus in pure culture it was transferred and grown on the following kinds of media: potato, lima bean, cornmeal, oatmeal, prune, nutrient, Czapek, and 0.2 per cent, 2 per cent and 5 per cent dextrose. It was grown also on sterilized cornmeal, wheat, oats, rice, lima beans, bean pods, wheat stems, wheat heads,

barley stems, barley heads, and sweet clover stems. The growth of the fungus was carefully studied on each of these media and a careful search was made for spore forms. Typical perithecia developed in from three to four months on the sterilized sweet clover and wheat stems.

Small amounts of the inoculum consisting of fungous growth on wheat kernels from one or more of these isolation strains were placed in 156 out of 234 pots of wheat at planting time. Steam-sterilized soil was used in the greater number of the pots. The results for a greater part of this test are included under the varietal tests of the host-range determinations. At maturity the plants in all of the 156 inoculated pots showed typical symptoms of take-all, while no plant in any of the 78 check pots exhibited such symptoms. The symptoms were similar in all respects to those obtained in other inoculation experiments in which bits of diseased straw taken from field plants were used as a source of inoculum, and also to those observed in the field. *O. cariceti* was reisolated several times from the discolored area of plants that had been inoculated with pure cultures, but it was never isolated from bits of the host plant taken above the discolored area at the base of the culm. The reisolated fungus agreed in every essential with the original isolations. Furthermore, the reisolated fungus, when used as an inoculum at planting time, caused typical symptoms of take-all on the growing wheat plants. There is no doubt, therefore, that the fungus *O. cariceti* is capable of causing typical take-all.

## DETERMINATION OF HOST RANGE

The need of a complete knowledge of the host range becomes at once apparent in considering the means of eliminating the take-all fungus from infected areas. Wheat and barley have been reported as being affected more often than rye and oats (23, 2, 31), which, however, are not immune. Rice also has been reported as subject to this disease in Japan (29).

The following grasses have been listed as hosts for the take-all fungus: *Agropyron repens, A. scaborum, Aira caespitosa, Bromus mollis, B. sterilis, Cynodon sp., Hordeum murinum* (1, 24, 16, 2, 31).

All varieties of wheat are considered as susceptible by Pridham (21). The percentage of infected plants among the eighty-one varieties which he tested varied from 1 to 33.7.

Workers in general agree that red wheats are more resistant than white wheats. According to Pridham (21), the early varieties are much freer from the disease than are the late varieties, but this does not agree with the statements of Lindau (14) and of the Great Britain Board of Agriculture (10). No record of the infection of spring wheat has been found by the writer.

*Field Hosts.* Winter wheat and winter rye are the only cereals on which take-all was found in 1920 and 1921. Winter wheat appeared to be the principal host of the take-all fungus.

The following varieties of soft winter wheat were found affected: Number 6 Junior, Dawson Golden Chaff, Red Rock, Leap Prolific, and Velvet Chaff. Field observations indicate that these varieties are all fairly susceptible, but Number 6 Junior and Leap Prolific were more severely affected than Dawson Golden Chaff and Red Rock. A trace of take-all was found in one field of Turkey Red, but since only two fields of this variety were examined definite conclusions cannot be drawn.

In 1921 forty-four spring wheat fields, twenty-five fields of oats, and seven barley fields were examined carefully but no take-all was found.

Rye appears to be resistant except when growing under unusual conditions. The only affected rye plant was found at Spencerport, New York, on June 23, 1921, in an area in a wheat field in which all of the wheat plants had been killed with take-all.

*Agropyron repens* was the only wild grass found affected with take-all. In infected areas in wheat fields this grass was very commonly affected with take-all in at least three New York counties: Cayuga, Genesee, and Monroe.

*Greenhouse Host Range Tests.* To determine the host range of take-all inoculation tests were made on sixty-two varieties of cereals and forty-eight species of grasses in the greenhouse. The cereals and grasses included in the test were planted on February 24, 1921. In the first part of this test a set of three five-inch pots were planted with seed of each variety of cereals or species of grass, using clean soil. No inoculum was placed in the soil of one pot of each set, but inoculum consisting of a mixture of four pure culture isolations of the causal organism was placed in the soil of the other two pots at the time of planting. The pure cultures were obtained from ascospore isolations, the inoculum being produced in quantity by growing the fungus in pure culture on steamed wheat in Erlenmeyer flasks. About ten of these wheat kernels were mixed with the soil before planting. Ten cereal seeds or from twenty-five to fifty grass seeds were planted in each pot.

At the end of ten and a half weeks nearly all of the wheat plants in the artificially inoculated pots were stunted, and 34.6 per cent had died. The discoloration at the base of the stem ranged from slight to that produced by fully formed plate mycelium. As shown in table 1, the percentage of killed plants varied greatly with the different varieties. No symptoms of discoloration or marked stunting of the plants were found for rye, oats, barley, corn, or most of the species of the grasses. However, a few of the species of grasses showed marked symptoms of stunting and discoloration.

TABLE 1

*Inoculation test on cereals*

| Variety (Wheat unless otherwise specified) | Percentage of plants dying during first 10½ weeks of growth | | Total number of heads formed per pot | | Relative number of perithecia on plants at maturity in inoculated pots | Degree of infection at maturity |
|---|---|---|---|---|---|---|
| | Inoculated pots | Uninoculated pots | Inoculated pots | Uninoculated pots | | |
| Kanred Ks 2401 | 34 | 0 | 0 | 3 | Many | Heavy |
| Kanred P 1066 | 37 | 0 | 1 | 11 | Many | Moderate |
| Kanred P 1068 | 41 | 0 | 1 | 4 | Moderate | Moderate |
| New Malakoff | 22 | 0 | Dead | + | Moderate | Very heavy |
| Turkey Red | 70 | 0 | 0 | 2 | Moderate | Moderate |
| Kharkof | 53 | 0 | 0.5 | 4 | Moderate | |
| Forward | 22 | 0 | 1.5 | 4 | Moderate | Heavy |
| Georgia Bluestem | 67 | 0 | | | | Moderate |
| Red Cross | 63 | 0 | 0 | 3 | Many | Heavy |
| Fultz | 26 | 11 | 0 | 0 | Many | Heavy |
| Number 8 | 14 | 0 | | | | Heavy |
| Poole | 56 | 0 | 0.5 | 4 | Many | |
| Red Wave | 10 | | 0.5 | 0 | Many | Heavy |
| Red May | 71 | | 0.5 | + | Many | Moderate |
| Fulcaster | 11 | 25 | 2 | 2 | Many | Moderate |
| Gypsy | 22 | 0 | 0 | 5 | Many | Moderate |
| Reliable | 11 | 0 | 0 | 5 | Many | Heavy |
| Red Rock | 0 | | 0.5 | 3 | Many | Heavy |
| Velvet Chaff | 25 | 0 | 0 | 5 | Many | Moderate |
| Crails Fife | 13 | 0 | 0 | 0 | Moderate | Heavy |
| Imperial Amber | 22 | | | | Many | Heavy |

TABLE 1 (Continued)

| Variety (Wheat unless otherwise specified) | Percentage of plants dying during first 10½ weeks of growth | | Total number of heads formed per pot | | Relative number of perithecia on plants at maturity in inoculated pots | Degree of infection at maturity |
|---|---|---|---|---|---|---|
| | Inoculated pots | Uninoculated pots | Inoculated pots | Uninoculated pots | | |
| Number 6 | 59 | 0 | 0 | 1 | Many | Heavy |
| Dawson Golden Chaff | 31 | | 0 | 4 | Many | Heavy |
| Klondyke | 25 | | 0 | 0 | Many | Moderate |
| Seneca Chief | 13 | 0 | 1 | 3 | Moderate | Moderate |
| S. S. Longberry | 12 | 0 | 0 | 0 | Many | Heavy |
| Genesee Giant | 22 | 0 | 0 | 2 | Many | Heavy |
| Early Arcadian | 6 | | All dead | | Many | Very heavy |
| Washington No. 108 | 0 | 0 | 1.5 | 15 | Many | Heavy |
| O. A. C. No. 104 | 9 | 0 | 0 | 4 | Many | Heavy |
| Little Club C. I.4066 | 25 | 10 | 0.5 | 1 | Many | Moderate |
| Bearded Club | 19 | 0 | 11 | 8 | Many | |
| Coffii | 6 | 0 | 0.5 | 6 | Many | Heavy |
| Marquis | 27 | 0 | 0 | 8 | Many | Heavy |
| Abysenia Purple Kernel | 16 | 0 | 1.5 | 10 | Many | Heavy |
| Coffee | 24 | 0 | 0.5 | 12 | Moderate | Moderate |
| Veivet Don | 93 | 0 | 0.5 | 9 | Many | Very heavy |
| Marovani | 60 | | 0.5 | 12 | Many | Heavy |
| Belotonka | 44 | 0 | 3 | 12 | Many | Heavy |
| Arnautka C. I. 1493 | 71 | 0 | Dead | 7 | Many | Very heavy |
| Acme | 89 | 0 | Dead | 8 | Many | Very heavy |

TABLE 1 (Continued)

| Variety (Wheat unless otherwise specified) | Percentage of plants dying during first 10½ weeks of growth | | Total number of heads formed per pot | | Relative number of perithecia on plants at maturity in inoculated pots | Degree of infection at maturity |
|---|---|---|---|---|---|---|
| | Inoculated pots | Uninoculated pots | Inoculated pots | Uninoculated pots | | |
| Mindum | 67 | 0 | 0 | 8 | Few | Heavy |
| Poulard | 53 | 0 | 1 | 10 | Many | |
| Polonicum | 29 | 0 | 2 | 12 | Moderate | Moderate |
| Black Persian | 59 | 0 | 0 | 10 | Many | Very heavy |
| White Beardless Spelt | 12 | 10 | 5 | 15 | Moderate | Moderate |
| Black Bearded Spelt | 21 | | 3 | 8 | Moderate | Moderate |
| Einkorn | 60 | 0 | 3.5 | 13 | Few | |
| Emmer C. I. 3686 | 60 | 0 | 0 | 8 | Many | Heavy |
| Black Winter Emmer | 17 | 0 | 0 | 5 | Many | Moderate |
| Alaska Branched | 0 | 0 | 0 | 10 | Many | Very heavy |
| Pelessier | 53 | 0 | 1 | 15 | Many | Heavy |
| Khapli | 36 | | 0 | 11 | Many | Heavy |
| Wild Wheat of Palestine | 61 | 0 | 0 | 5 | Many | |
| Barley 6 row | 0 | 0 | + | + | Many | Moderate |
| Malay Beardless | 0 | 0 | 2 | 10 | Very many | Moderate |
| Barley (C. I. 1176 Oderbrucker | 6 | | 3 | 15 | Very many | Moderate |
| Barley (C. I. 537) Oats | 0 | 0 | 12 | 14 | None | None |
| Rye Winter | 10 | 0 | 0.5 | 1 | Very few | Slight |
| Rye, Rosen | 0 | 0 | — | — | None | None |
| Field corn | 0 | 0 | — | — | None | None |
| Sweet corn | | | | | | |

Perithecia had formed in ten and one-half weeks after planting on the following plants in the artificially inoculated pots: winter wheats—Dawson Golden Chaff, Number 6 Junior, Seneca Chief, O. A. C. No. 104: spring wheats—Little Club, Einkorn, Velvet Don: grasses—*Agropyron repens, Bromus madritensis, Elymus virginicus.* Fully developed ascospores were found in the perithecia on Seneca Chief and O. A. C. No. 104 wheat.

The time taken by the plants to reach maturity varied from about four months for the spring cereals and the annual grasses, to seven months for the winter cereals and many of the perennial grasses.

The grains in the heads of the wheat plants growing in soil infected with the fungus were always very badly shriveled. The total grain produced by such plants weighed less than five per cent of the weight of that in heads of normal plants.

The relative number of perithecia on the affected plants varied from none to many. The term few means less than ten perithecia per culm; moderate, from ten to forty perithecia; and many, more than forty perithecia. Over one hundred perithecia were counted on single culms of Number 6 Junior, Velvet Don, and Red Rock wheat.

In the greenhouse no variety of the many types of wheat showed any marked degree of resistance to take-all, but the limitations of such experiments must be considered.

The three barley varieties tested showed no symptoms of take-all during the first ten and a half weeks of growth, but at the end of four months, when the barley was mature, the mycelial plate was even more pronounced than on wheat, and perithecia were as numerous as on any wheat variety. The characteristic stunting of the wheat plants seemed to be lacking in the case of the barley.

During the first four months of growth no symptoms of the disease appeared on rye. After six months, however, an indistinct plate mycelium and a few perithecia containing typical ascospores had developed on plants of Rosen rye. The results of inoculating forty-eight species of grasses are given in table 2.

Since the most important rôle of the grasses would be as carriers of the disease the chief object of this test was the determination of the grasses on which perithecia were produced. In order to determine this numerous culms of each species of grass were picked to pieces under the binocular, and where perithecia were present (Table 2) the presence of typical ascospores was demonstrated.

TABLE 2

*Inoculation tests on grasses. Condition of grasses from five to five and one-half months after planting seed in soil infested with O. cariceti.*

| Name of grass | Presence of mycelium | Relative number of perithecia on plants in inoculated pots | Degree of infection |
|---|---|---|---|
| Agropyron caninum (L.) Beauv..... | Heavy | Many | Moderate |
| " cristatum J. Gaert..... | Slight | Few | Moderate |
| " intermedium Beauv..... | Heavy | Many | Heavy |
| " repens (L.) Beauv..... | Heavy | Many | Very heavy |
| " smithii Rydb..... | Slight | Few | Slight |
| " tenerum Vasey..... | Heavy | Many | Moderate |
| Agrostis alba L..... | | 0 | 0 |
| " canina L..... | | 0 | 0 |
| Alopecurus pratensis L..... | | 0 | 0 |
| Arrhenatherum elatius (L.) Beauv... | | 0 | 0 |
| Anthroxanthum odoratum L..... | | 0 | 0 |
| Avena fatua L..... | | 0 | 0 |
| Briza maxima L..... | | 0 | 0 |
| Bromus arvensis L..... | Slight | Few | Slight |
| " ciliatus L..... | Slight | Few | Slight |
| " erectus Huds..... | Slight | Moderate | Moderate |
| " hordeaceus L..... | | 0 | 0 |
| " inermis Leyss..... | | 0 | 0 |
| " madritensis L..... | Slight | Moderate | Slight |
| " japanicus Thunb..... | Moderate | Moderate | Moderate |
| " racemosus L..... | Moderate | Moderate | Moderate |
| " secalinus L..... | Moderate | Many | Slight |
| " sterilis L..... | Moderate | Many | Moderate |
| " tectorum L..... | Moderate | Many | Moderate |
| " villosus Forsk..... | | 0 | 0 |
| Dactylis glomerata L..... | | 0 | 0 |
| Echinochloa crusgalli (L.) Beauv..... | | 0 | 0 |
| Elymus australis Scribn. & Ball..... | Moderate | Many | Moderate |
| " canadensis L..... | Heavy | Many | Heavy |
| " virginicus L..... | Moderate | Many | Moderate |
| Festuca elatior L..... | Slight | Few | Slight |
| " heterophylia (Lam.) Hack.. | | 0 | 0 |
| " ovina L..... | | 0 | 0 |
| " pratensis Huds..... | | 0 | 0 |
| " rubra L..... | | 0 | 0 |
| Holcus lanatus L..... | | 0 | 0 |
| Hordeum jubatum L..... | Heavy | Many | Heavy |
| " murinum L..... | Slight | Few | Slight |
| " pusillum Nutt..... | | 0 | 0 |

TABLE 2 (Continued)

| Name of grass | Presence of mycelium | Relative number of perithecia on plants in inoculated pots | Degree of infection |
|---|---|---|---|
| Hystrix patula Moench | Moderate | Many | Heavy |
| Lolium perenne L. | | 0 | 0 |
| " temulentum L. | Slight | Few | Slight |
| Phlaris arundinaceae L. | Moderate | Many | Moderate |
| Phleum pratense L. | | 0 | 0 |
| Poa compressa L. | | 0 | 0 |
| " pratensis L. | | 0 | 0 |
| Sataria glauca (L.) Beauv. | | 0 | 0 |
| Trioda flavus (L.) Hitche) | | 0 | 0 |

The number of perithecia on each culm of the various species ranged from one to three in the case of *Festuca elatior*, to as high as one hundred and eight found on one culm of *Agropyron repens*. The terms, few, moderate, and many given in table 2, have the same meaning as in table 1.

The presence of mycelium refers to discoloration at the base of the culm of the infected plants. No definite plate of mycelium was found in the case of the grasses between the inner leaf and the culm. Rather numerous darkened strands of hyphae were formed in and between the several leaf sheaths.

A second series, in which five cereal varieties and fifteen grass species were used, was planted at the same time as the first in unsterilized soil taken from a diseased spot in a field and mixed with bits of diseased straw. The conditions under which this series was grown were the same as those of the first, except as stated. These plants developed typical take-all symptoms later in their growth than did those grown in soil infested with a pure culture of *O. cariceti*. However, at maturity the plants of the second series very closely resembled those grown in artificially inoculated soil in the degree of stunting, the presence of darkened tissue and plate mycelium, the relative number of perithecia, and the range of the species of grasses on which perithecia were found.

## REPRODUCTION AND DISSEMINATION OF THE FUNGUS

### FORMATION OF SPORES

Examination of field and greenhouse material, supplemented by examination of the fungus growing in pure culture on many kinds of media, confirms Lindau's statement (14) that no conidial form of the

fungus is definitely known. Healthy and diseased wheat plants have grown side by side for ten months in pots set in the soil of a greenhouse bench without the healthy plants becoming infected. If a conidial stage exists, it apparently plays no rôle in the dissemination of the fungus.

Sexual spores, however, are produced abundantly. From one to more than a hundred perithecia were formed on almost every diseased culm of wheat examined. Perithecia containing differentiated ascospores were found on winter wheat as early as June 5, from four to six weeks before the maximum number had been produced. In June and July the ascospores appear to be immature: they fail to germinate, they are from 10 to 20μ shorter than those examined later in the year, and they are rarely, if at all, septate. Ascospores formed on winter wheat in June and July of 1920 failed to germinate when tested in July and August. Ascospores from the same source kept under field conditions, germinated profusely on October 20, November 10, November 16, and December 3, 1920, and on January 19 and March 26, 1921, but failed to germinate on August 20, 1921. This would indicate that under field conditions the ascospores can germinate and may infect their hosts either during the fall or the following spring.

### METHOD OF DISSEMINATION OF THE FUNGUS

The literature gives little information as to how Ophiobolus is carried from one locality to another. It has been pointed out by Waters (31) that infective material may be carried for a short distance on the feet of animals and on cultivators. Observations made thus far in the field indicate that the pathogene is disseminated commonly by infected straw in manure. McAlpine (16) believes that dust or wind-blown soil is responsible for much of the distribution of the fungus in parts of Australia, although this probably would occur less frequently in the humid regions of the United States. Waters (31) states that in New Zealand "no instance could be found of the rapid spread of take-all from crop to crop through the air like rust." Since most of the ascospores are produced near or even below the ground level, it does not seem likely that wind is an important factor in the dissemination of the fungus under our conditions.

Many growers rake over the stubble after the binder. The material thus collected goes with the bulk of the harvest to the thresher. The fungus may thus be spread either with the thresher or with improperly cleaned seed.

The series of tests tabulated below were planned to determine whether seed, soil, or plant residue acted as a carrier of the fungus. The plantings were made at different dates, as given in the tabulations. From ten to fifteen seeds of Number 6 Junior or Dawson Golden Chaff were

planted in each pot. The pots which were five-inch size, were set four inches in the soil of a greenhouse bench where they remained throughout the test. Unsterilized bench soil was considered as being clean soil. The infested soil used came from a spot in a field in which diseased plants had been grown, and was carefully screened in order to remove bits of infected tissue that might have been present. The inoculum in every case was mixed with the soil when the seed were planted.

*Shriveled seed from diseased plants. Clean soil. No prepared inoculum*

| | | |
|---|---|---|
| July 31, 1920 | 2 pots | Plants exhibited no symptoms of infection and grew to maturity producing normal heads. |
| September 13, 1920 | 1 pot | |
| October 4, 1920 | 4 pots | |

*Shriveled seed from diseased plants. Soil from infected spot in field*

| | | |
|---|---|---|
| July 31, 1920 | 4 pots | All plants stunted and many killed. Perithecia on all. Infection heavy. |
| September 13, 1920 | 2 pots | |
| October 4, 1920 | 5 pots | |

*Clean seed. Clean soil. No prepared inoculum*

| | | |
|---|---|---|
| July 31, 1920 | 2 pots | Plants exhibited no symptoms of infection and grew to maturity producing many normal heads. |
| September 13, 1920 | 2 pots | |
| October 4, 1920 | 2 pots | |
| Mar. 26, 1921 | 3 pots | |

*Clean seed. Soil from infected spot in field. Soil had been stored indoors since July, 1920*

| | | |
|---|---|---|
| October 4, 1920 | 5 pots | Plants in two of the five pots diseased, those in the other three pots showing no sign of infection. |
| March 26, 1921 | 4 pots | Plants somewhat stunted but exhibiting no typical symptoms. No perithecia found. |

*Clean seed. Clean soil. Bits of diseased straw containing perithecia used as inoculum*

| | | |
|---|---|---|
| July 31, 1920 | 2 pots | All plants stunted and killed, exhibiting typical symptoms. Many perithecia Infection very heavy. |
| March 26, 1921 | 3 pots | |

*Clean seed. Soil from diseased spot in field. Bits of diseased straw containing perithecia used as inoculum*

| | | |
|---|---|---|
| September 13, 1920 | 4 pots | All plants stunted and killed, exhibiting typical symptoms. Many perithecia. Infection very heavy. |

These tabulations indicate that seed from diseased plants do not act as carriers of the disease; that carefully screened soil from infected spots in the field acts as inoculum for a time, but that such soil when stored indoors for a period of eight months no longer contains infective material

and that bits of straw containing perithecia are the most virulent inoculum used, the virulence of this inoculum being undiminished at the end of eight months as was the case with the soil. Further, since the healthy plants grew side by side with diseased plants throughout the test without becoming infected, it seems that the soil was the principal source of inoculum.

### SOME FACTORS INFLUENCING THE GROWTH OF THE FUNGUS

Many investigators have attempted to influence the growth of the fungus by the application of various fertilizers and other substances. Lime has generally been reported as greatly increasing the amount of disease (2). Superphosphate of lime has been reported as increasing (2), as not checking (18), and as decreasing (10) the growth of the fungus. Copper sulphate in some cases has had no effect (9), and in other cases has caused a marked reduction in growth (3). The application of from 100 to 1000 pounds of iron sulfate per acre has generally checked the growth of the fungus (5, 3). From these more or less conflicting reports, it seems that the addition of alkali favors the fungus, while acid seems to retard it.

To test the effect of certain fertilizers in a preliminary way the following experiment was run in the greenhouse: Twenty-five five-inch pots were filled with clean soil and were inoculated at the same time and under the same conditions as those of the first part of the host-range test, except that five kernels of Number 6 Junior wheat were planted in each pot. The twenty-five pots were divided into five series of five pots each. The first series of pots received no fertilizer, while each of the other four series received one of the following fertilizer treatments:

1. One gram of sodium nitrate per pot, applied by dissolving in 100 cc. of water which was poured over the soil.
2. One-half gram of acid potassium phosphate (diabasic) per pot. Applied by mixing with soil.
3. One gram of sulphur per pot. Applied by mixing with soil.
4. Three grams of hydrated lime per pot.

After seventeen weeks of growth, no marked differences were found among the different series. Later, the series to which lime had been added was the first on which perithecia were found, and this series had more killed plants (15.7 per cent) than the sodium nitrate series (13.6 per cent), the acid potassium phosphate series (5 per cent), or the sulphur series (none) and had less dead plants than the series to which no fertilizer had been added (25 per cent).

After eight months of growth, the plants in all of the series except the one to which sulphur had been added were heavily infected, were

badly stunted, and had produced no heads. Even the plants in the sulphur series were heavily infected, but those in three of the pots succeeded in producing several heads. If anything, acidity of soil seemed to have delayed the attack of the fungus or lessened its severity.

In order to further test this point, the fungus was grown in culture on an acidity series consisting of potato agar having nineteen degrees of acidity ranging from 3.2 to 9.6 pH, and bacto-cornmeal agar having ten degrees of acidity ranging from 3 to 9.2 pH. The degree of acidity of the media was determined by the colorimeter method as recommended by Clark (4).

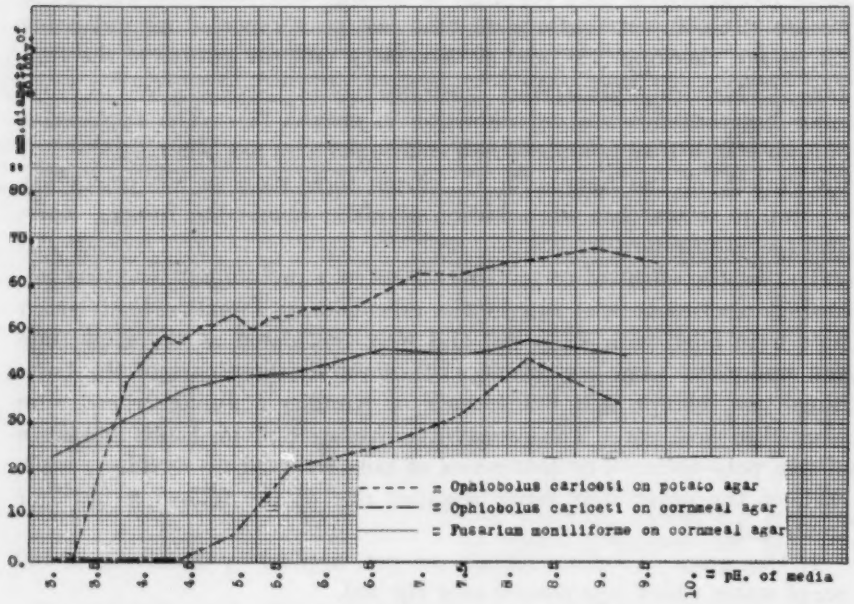
= Ophiobolus cariceti on potato agar
= Ophiobolus cariceti on cornmeal agar
= Fusarium moniliforme on cornmeal agar
= pH. of media

FIG. 3. RATE OF GROWTH OF *Ophiobolus cariceti* AND *Fusarium moniliforme* ON AGAR HAVING VARYING DEGREES OF ACIDITY

Cultures of *O. cariceti* from three sources, in comparison with one strain of *Fusarium moniliforme* Sheld., were grown on the media in total darkness at room temperature (15 to 20° C.) The average growth of the three strains of *O. cariceti* and the growth of the single strain of *F. moniliforme* at the end of eight days are shown in figure 3.

The results indicate that the fungus requires alkalinity for optimum growth, and may explain why additions of alkaline substances to the soil have been reported as favoring the fungus while additions of acid-forming substances decrease the amount of infection. The writer now has under way several series of greenhouse and field tests to determine the effect of soil acidity on the resistance of the hosts to the fungus.

Several investigators (2, 7) point out that excessive soil moisture is favorable to the growth of the fungus. For this reason, careful observations were made of the relation of soil moisture to the severity of the disease. In most fields examined, apparently there was little or no difference in the amount of infection on high and low ground, although in a few fields the infection appeared to be very much heavier on the lower and wetter land.

## CONTROL

Many suggestions appear in the literature concerning the control of take-all, among the most common of which are: eliminating the fungus by burning the diseased stubble and by eradicating wild grass and volunteer grain which act as hosts (16, 25, 28); practicing long rotations, using crops believed to be immune (16, 23, 31); retarding infection by the addition of certain substances to the soil such as phosphates and sulphates, which either check the growth of the fungus or so strengthen the cereal plants that they may become resistant (27, 5, 10); proper drainage of the soil (2, 7); escaping infection by the practice of late planting (28, 31); preventing infection by the use of resistant varieties (10, 21). The statements in regard to some of these methods are so conflicting as to place their value somewhat in doubt.

Rotation appears to be an important method of control, since in New York State it was observed that nearly every field in which more than five per cent of the plants were killed by take-all had been planted to two or more successive crops of wheat. A rotation, to be effective, should have wheat not oftener than every four or five years, since the fungus can live in the stubble for at least a year, and several years of cropping may be required for the fungus to die out on volunteer grain or other hosts.

Diseased wheat stubble is the principal carrier of the take-all fungus. Therefore, straw containing plants broken or cut off low enough to make them carriers of the organism, should not be returned to the land in manure or in any other way for at least three years preceding the planting of wheat. It was observed that wheat straw from diseased fields, when applied in manure during the fall and winter of 1920–21, acted as a direct carrier of the fungus.

Wheat seed should be thoroughly cleaned before planting. The mycelium does not seem to be carried inside the seed, nor do the spores seem to be carried on the seed coat, but bits of straw containing perithecia may become mixed with the threshed grain and the pathogene might then be carried with the seed. It would be well not to lime the soil before planting wheat, since acidity retards and alkalinity promotes the growth of the take-all fungus. Other suggested methods of control are late planting and the use of resistant varieties, although definite recommendations cannot be made on these two points.

SUMMARY

1. The take-all disease of cereals and grasses caused by the fungus *Ophiobolus cariceti* (B. & Br.) Sacc. was first found in New York in 1920. A survey made in 1921 demonstrated that the disease was present in nearly one half of the winter wheat fields of sixteen counties in the west central part of the state.

2. The disease was comparable in severity to that described in Australia and Europe. The average damage in the seventy-eight fields found infected in 1921 was about two per cent, and the maximum damage in any one field was about twenty per cent.

3. The most typical symptom of the disease is a dwarfing of the host, which includes a reduction in height, in the number of tillers, in the number of heads, and in the size and amount of grain produced. The yield of an infected wheat plant was, on the average, about one per cent that of a healthy plant.

4. The fungus is confined to the roots and the lower internodes of the host, where a pronounced discoloration occurs. A typical plate of mycelium is found between the leaf sheath and the culm, and perithecia are produced in abundance, more than one hundred having been found on single culms of wheat and of *Agropyron repens*.

5. As the results of inoculations in the greenhouse, typical perithecia were produced on wheat, barley, rye, and one or more species of the following genera of wild grasses: Agropyron, Bromus, Elymus, Festuca, Hordeum, Hystrix, Lolium, Phalaris.

6. None of the fifty-four varieties of wheat tested in the greenhouse showed any marked degree of resistance to take-all. These varieties included representatives of the following species of the genus Triticum; *aestivum*, *compactum*, *turgidum*, *durum*, *dicoccum*, *spelta*, *polonicum*, and *monococcum*.

7. The causal organism was isolated and grown in pure culture on numerous media. Typical perithecia have been produced in pure culture.

8. In the present investigation, seed from diseased plants did not act as carriers of the disease. Screened soil from infected spots in fields acted as inoculum for several months, but at the end of eight months the soil which had been kept in the laboratory was not a carrier of the disease. Bits of infected straw containing perithecia were very effective inocula, and in this case the virulence of the organism had not decreased at the end of eight months.

9. *O. cariceti* requires a condition of alkalinity for optimum growth. On cornmeal agar, growth begins at about 4.5 pH. and increases gradually to 8.1 pH, the point at which maximum growth occurs.

10. In the present investigation, the relation of *O. cariceti* to take-all was determined by the established rules of proof.

11. The most promising methods of control seem to be: the practice of four to five year rotations; eradicating wild grass and volunteer grain which may act as hosts; discontinuing the practice of returning wheat stubble in manure for three years preceding the planting of wheat; cleaning the wheat seed thoroughly to remove all bits of straw which might carry perithecia; and discontinuing the practice of liming the soil before planting wheat.

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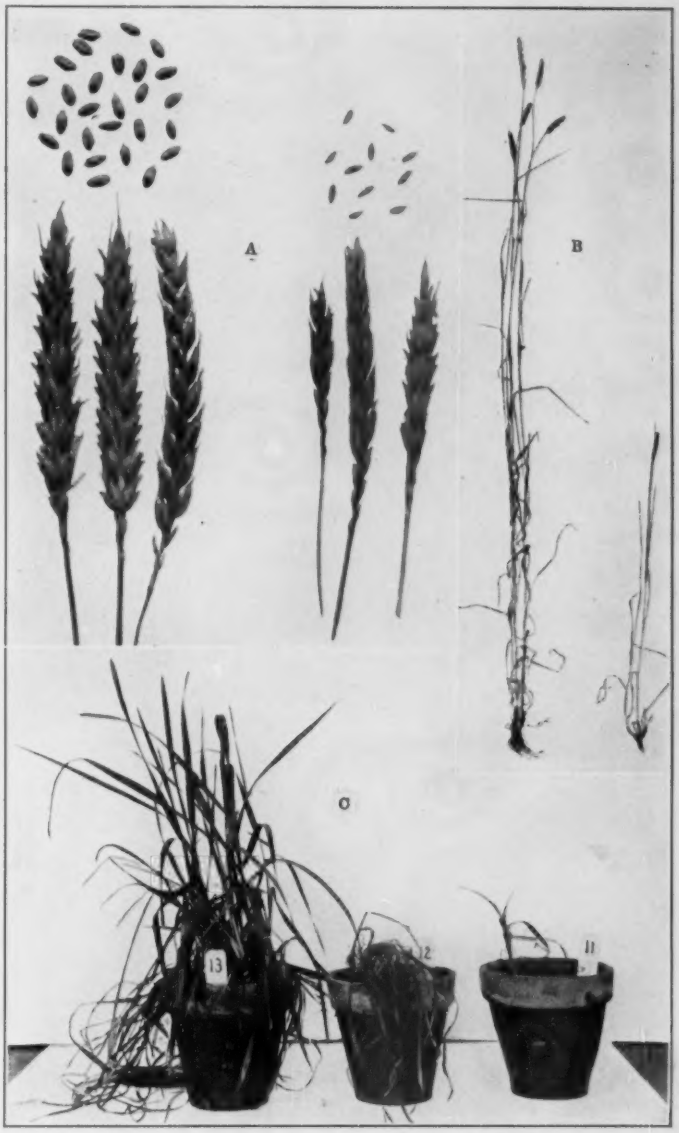


TAKE-ALL DISEASE OF CEREALS AND GRASSES

FIG. A. Heads taken from healthy (left) and take-all infected (right) wheat plants.

FIG. B. Healthy and take-all infected wheat plants. On left, healthy plant with five heads, height 40 inches. On right, diseased plant with one shriveled head, height 18 inches.

FIG. C. Healthy and diseased plants after four months' growth.

Pot 13. Check.

Pot 11–12. Diseased straw mixed with soil at planting time.

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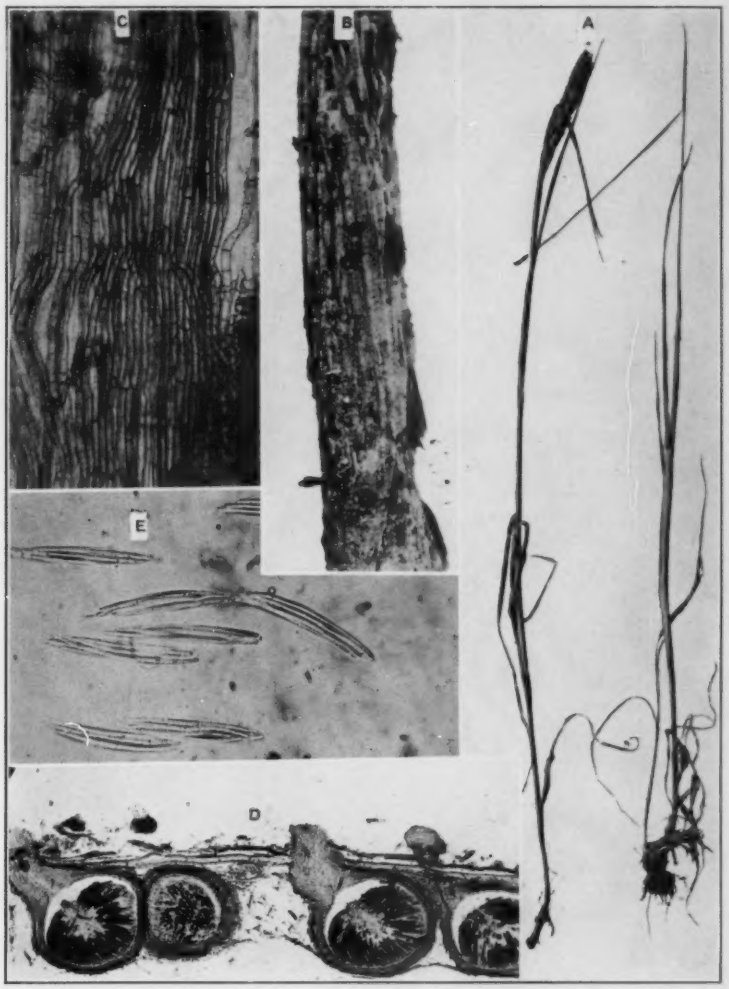


TAKE-ALL DISEASE OF CEREALS AND GRASSES

FIG. A. Two wheat plants (height 16 in.) infected with take-all taken from a field at harvest time. The marked stunting of the plants is characteristic of this disease.

FIG. B. Base of wheat plant infected with take-all showing beaks of perithecia protruding through the leaf sheaths. x 4

FIG. C. A portion of the mycelial plate found about the base of the culm. x160

FIG. D. Longitudinal section through the outer leaf sheath of a wheat plant showing structure and contents of perithecia. Note the beaks of the perithecia protruding obliquely through the leaf sheath. x 48

FIG. E. Asci and ascospores. x 160

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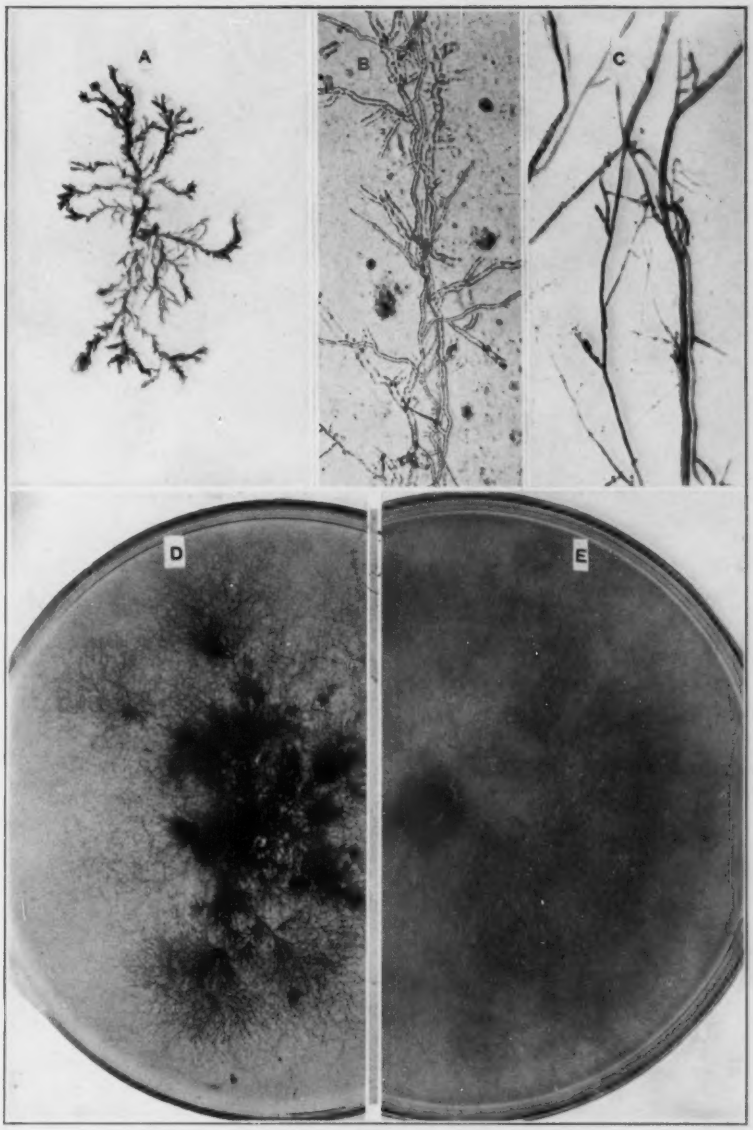


TAKE-ALL DISEASE OF CEREALS AND GRASSES

FIG. A. Thirty days old culture of *O. cariceti* on 0.2 per cent dextrose agar x 9

FIG. B. Characteristic rhizomorphic strands of mycelium as found on 2 per cent dextrose agar after 30 days' growth. x 450

Fig. C. Characteristic ribbon-like rhizomorphic bands of mycelium as found on 2 per cent dextrose agar after 30 days' growth. x 450

FIG. D. Thirty days' old culture of *O. cariceti* on 2 per cent dextrose. x 9

FIG. E. Thirty days' old culture of *O. cariceti* on potato agar. x 9

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# STUDIES ON CORN RUST

GEORGE F. WEBER[1]

WITH THREE FIGURES IN THE TEXT

## INTRODUCTION

In connection with studies on corn rust (*Puccinia sorghi* Schw.) conducted at the University of Wisconsin during the year 1919–1920, data were obtained particularly on: (1) the relation of temperature to germination of urediniospores; (2) the relation of temperature to infection by urediniospores; (3) mode of host penetration in uredinial infection; (4) overwintering of urediniospores; (5) relative susceptibility of corn species. Corn seedlings were grown in the greenhouse in eight-inch pots and in flats six inches deep. Urediniospores were obtained from infected plants in the greenhouse, except when otherwise stated. Inoculations were made by rubbing the corn leaves with wet absorbent cotton after which the spores were gently applied with a scalpel or platinum loop. All inoculated plants were incubated in a moist chamber for thirty-six hours immediately following inoculation. In the course of the studies it was noted that when urediniospores were shaken from pustules their percentage of germination was higher than when the spores were removed with a scalpel. Hence in order to collect uninjured spores of the same stage of maturity for the experiments, a medicine dropper was used as follows: A droplet of water was squeezed from the dropper and gently stroked over the top of the pustule, the mature spores were picked up readily by the surface of the drop.

## EXPERIMENTAL STUDIES

### THE RELATION OF TEMPERATURE TO GERMINATION OF UREDINIOSPORES

In germinating rust spores Johnson (3) found no uniformity between the temperatures of 7° and 25° C. Melhus (6) determined the optimum temperature for the germination of urediniospores of *Puccinia sorghi* Schw. at 18° C. Mains (5) found 15°–18° C. to be the optimum and 25° C. the maximum temperatures for urediniospore germination.

[1] The writer wishes to express his sincere appreciation to Dr. A. G. Johnson for suggesting the investigation of this problem and for assisting in the preparation of the manuscript; to Dr. J. G. Dickson for the many valuable suggestions and helpful criticisms during the progress of the work; and to Mr. J. R. Holbert for furnishing the seed of the different kinds of corn used in these experiments and for access to his experimental plats for the purpose of making observations.

Mature urediniospores collected by the writer in the manner stated above were placed in hanging drops on the covers of petri dishes, the bottoms of which were covered with water and placed at different temperatures in an Altmann graduated incubator. At the end of twenty-four hours counts were made of germinated and ungerminated spores. The average percentages of germination of twelve series are given in table 1 and shown graphically in figure 1. In each series from four to twelve drop cultures were observed for each temperature.

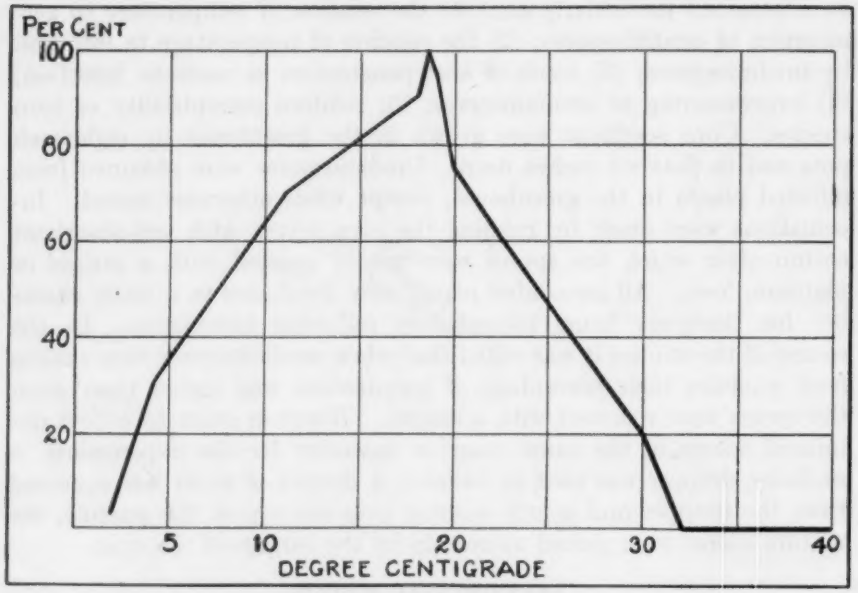


FIG. 1. GRAPH SHOWING PERCENTAGES OF GERMINATION OF UREDINIOSPORES OF CORN RUST AT DIFFERENT TEMPERATURES

TABLE 1

*Temperatures of incubation and percentages of germination of urediniospores of corn rust after 24 hours*

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature in degrees C | 4 | 8 | 12 | 16 | 17 | 18 | 20 | 25 | 30 | 34 | 40 |
| Germination | 3 | 30 | 70 | 90 | 100 | 95 | 75 | 60 | 20 | 0 | 0 |

It will be noted that the highest percentage of germination resulted at 17° C., and that germination diminished rapidly toward the lower and higher temperatures.

At 8° C. the germ tubes were short, thick, very definite in outline and appeared turgid. At the tips of the germ tubes the protoplasm was denser than toward the spore. In contrast with this at 30° C. the germ tubes were long, narrow, hyaline, thin-walled, without visible protoplasm, and showed some lack of turgor.

### THE RELATION OF TEMPERATURE TO INFECTION BY UREDINIOSPORES

A series of corn seedlings fifteen days old were inoculated and placed in temperature chambers regulated at 8°, 14°, 18°, 25° and 32° C. respectively. The humidity was not controlled but it was kept close to saturation. The results tabulated in table 2 are given in percentage of infection after twelve days according to a scale of comparison adopted by the Office of Cereal Investigations, United States Department of Agriculture, and published by Durrell and Parker (2).

TABLE 2

*Percentage of infection on corn plants inoculated with urediniospores of Puccinia sorghi and incubated at different temperatures for twelve days*

| Temperature in degrees C. | 8 | 14 | 18 | 25 | 32 |
|---|---|---|---|---|---|
| Percentage of infection | 29 | 50 | 90 | 20 | 0 |

The data in table 2 show that the highest percentage of infection took place at 18° C, and that infection gradually decreased toward the higher and lower temperatures respectively. The urediniospores did not germinate at 32° C., and at 30° C. they showed only a slight tendency toward germination, but at this temperature they did not infect the corn plant. Infection took place at 28° C. but the fungus did not develop well. The rate of development of the fungus within the host was most rapid at 20° C.; at lower temperatures its growth was slower.

### MODE OF HOST PENETRATION IN UREDINIAL INFECTION

The second and third leaves of corn seedlings, ten days old, growing in 8-inch pots, were inoculated with mature urediniospores. The inoculated plants were placed in a moist chamber and, successively after twenty-four, thirty-six and forty-eight hours, pieces 1 cm. square were cut from the inoculated leaves. These pieces were placed in a solution of equal parts of glacial acetic acid and 95 per cent alcohol for twenty-four hours. By this method the cells were killed, fixed and cleared. They were then stained *in toto* with Pianese IIIb stain, as used by Vaughan (7), and examined under the low power of the microscope. The host tissue stained green and the fungus rose.

When the surface of the leaf was moist the germ tubes extended in all directions, when dry they showed a marked tendency to follow the sunken furrows between the epidermal cells. It was also shown in these studies that there was no attraction of the germ tube toward the stomata, although one was never observed growing across the stomatal opening without entering. Usually an appressorium was formed over the stoma, often almost covering it. Then it sent out a slender thread-like hypha into the stomatal chamber, which developed into mycelium (Fig. 2a). However, not all germ tubes develop appressoria before entering the stomata (Fig. 2b). If the stomata were open the germ

tubes entered immediately. The mycelium was developed in the intercellular spaces in contact with the parenchymatous cells of the host.

## OVERWINTERING OF UREDINIOSPORES

In reviewing the literature on corn rust no data, based on experimental evidence, were found concerning the overwintering of urediniospores. However, Carlton (1) observed that the urediniospores of *Puccinia sorghi* Schw. did not remain viable over winter. Kellerman

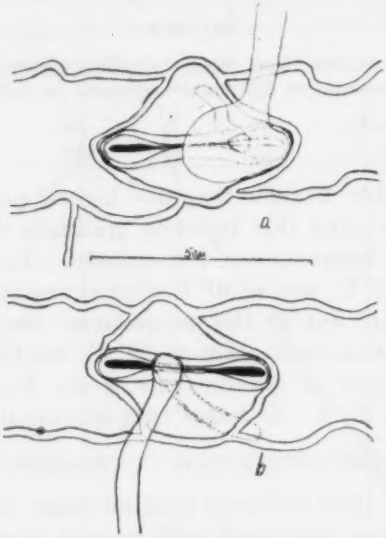


FIG. 2. CORN RUST ENTERING STOMATA OF CORN LEAF.

a. Hypha from urediniospore formed appressorium over one end of stoma and penetration tube from appresorium entered opening of stoma.

b. Hypha from urediniospore entered opening of stoma without forming appressorium.

(4) stated that surviving urediniospores carried the disease over winter. This lack of data prompted the writer to conduct a series of experiments during the winter of 1919–1920, at Madison, Wisconsin, for the purpose of determining the viability of urediniospores from the time of their maturity in September until May of the following year. Rusted corn leaves were collected before frost and carefully dried at room temperature. They were then placed at ten different stations under somewhat varied environmental conditions. These stations were designated by the numbers 1–10. Stations 1 to 4 were on the bare ground on the four sides of a hill, namely, north, east, south and west sides respectively. Stations 5 and 6 were on the north and south sides respectively of a greenhouse. Stations 7 to 9 were corn leaves suspended

at different heights from the ground on the side of a building. At station 10 the corn leaves were slightly covered with soil. All germination tests were made at 18° C. Germination tests were made just before the rusted leaves were placed at the different stations, October 1, 1919, and every two weeks thereafter until April 30, 1920. All the germination tests were made in distilled water by placing the spores in hanging drops in petri dish covers as previously described and incubating in the dark. The average percentages of germination at the different stations at the different times are given in table 3.

The results are given in percentage in all cases except where "S" follows the figure, in which cases the figure indicates the number of spores germinating on the whole petri dish cover. At first, 18 hours were sufficient for germination, but as the spores became less viable, 24 hours and finally 48 hours were required for the germination test.

TABLE 3

*The average percentages of the germination of urediniospores collected from the different stations from October 1, 1919, to April 30, 1920*

| Date | Percentage germination of spores | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Station | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Oct. 1 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 100 |
| Oct. 15 | 90 | 70 | 90 | 85 | 90 | 80 | 90 | 100 | 90 | 95 |
| Nov. 1 | 90 | 40 | 85 | 70 | 70 | 60 | 60 | 95 | 75 | 75 |
| Nov. 15 | 80 | 10 | 60 | 40 | 30 | 25 | 10 | 80 | 25 | 14 |
| Dec. 1 | 75 | 3S | 75 | 5 | 0 | 1 | 45 | 6 | 20 | 0 |
| Dec. 15 | 50 | 1 | 20 | 5 | 5 | 2 | 5 | 1 | 5 | 1S |
| Jan. 1 | 5 | 0 | 2 | 0 | 7 | 5 | 0 | 0 | 1 | 1 |
| Jan. 15 | 5 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 |
| Feb. 1 | 0 | 1S | 0 | 1 | 0 | 0 | 0 | 0 | 4S | 0 |
| Feb. 15 | 2S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mar. 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mar. 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apr. 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apr. 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apr. 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

It will be noted that there was a high percentage of germination during the first 30 days, followed by a rapid decline in germination from 40 to 60 days after the first tests were made. Coincident with this decline in germination the normal appearance of the germ tubes changed. They became more hyaline, thin-walled, and showed some lack of turgor.

It will be seen from table 3 that good germination resulted up until December 1, 1919, when for the first time no germination was observed from certain stations. On January 1 the urediniospores from other stations showed no germination. On January 3 urediniospores were collected from the ten stations and used as separate inocula on twelve day old corn plants in the greenhouse. These corn seedlings were in the fourth leaf stage. From each collection a spore suspension was made and the third leaf of five different corn plants was inoculated in

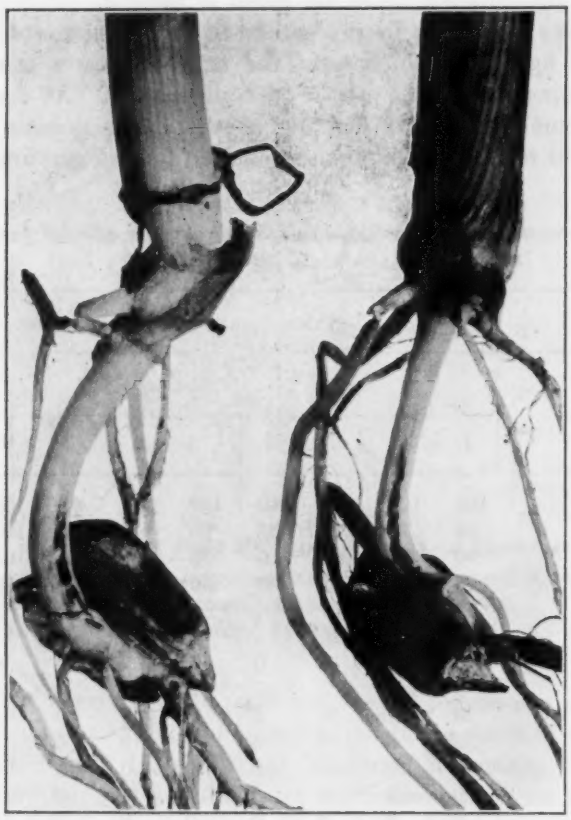

FIG. 3. UREDINIA OF CORN RUST ON MESOCOTYLS OF DENT CORN FOLLOWING ARTIFICIAL INOCULATION OF GERMINATING KERNEL.

the usual way. These plants were incubated in a moist chamber at 20° C. for 48 hours and then removed to a greenhouse at 24° C. No infection resulted from any of these inoculations. The check plants similarly inoculated and incubated, except with fresh urediniospores, showed abundant uredinia containing mature spores. After eight days four other similar series of tests were conducted with the same results,

i. e., no infection except where fresh spores were used. The inoculated plants were kept under observation for 20 days. Hence it was evident that the spores were either dead or had lost their pathogenicity.

In connection with the overwintering studies, certain attempts were made to determine if soil or seed borne infection were at all possible. Corn seeds were germinated in moist chambers until the coleoptile and radicle were about one-half inch long. They were then submerged in a urediniospore suspension for five minutes and immediately planted two inches deep in sterilized soil. After four weeks the soil was washed from the corn roots and uredinia containing mature spores were found on the mesocotyls of the seedlings one and one-half inches below the surface of the soil. Figure 3 shows the uredinia on the mesocotyls of two of these corn seedlings.

While uredinia were obtained in this way, it was not shown that they could function in initiating rust infections on the above-ground parts. Under certain conditions, however, this might not be impossible.

## RELATIVE SUSCEPTIBILITY OF CORN SPECIES

In order to determine the relative susceptibility of corn species it was found necessary to establish a strain of rust to be used throughout the series of experiments. This was done as follows: Rusted leaves had been collected by the writer from sixty-three different species and varieties of corn, grown in an experimental plat at Bloomington, Illinois, by J. R. Holbert. In preliminary experiments urediniospores from each of these collections were used to inoculate corn seedlings of the seven species and six varieties. For each set of inoculations the corn seedlings used were grown in greenhouse flats 16x24x4 inches. It was found that all of the spore collections behaved alike on each of the species and varieties tested. However, on the various species and varieties there was a rather constant range in susceptibility to the rust from the different sources. Hence no evidence of specialization in the rust was found.

On the basis of the above results urediniospores grown in the greenhouse on various species and varieties of corn were used in subsequent tests of relative susceptibility. In testing the relative susceptibility the following seven species of corn were used: *Zea everta* (pop); *Z. indurata* (flint); *Z. amylacea* (flour); *Z. saccharata* (sweet); *Z. indentata* (dent); *Z. tunicata* (pod); and *Z. ramosa*. These corn species were grown in flats in the greenhouse as previously described. When the seedlings had developed their second leaves they were inoculated with fresh, mature, urediniospores developed on greenhouse plants. The inoculated plants were then placed in a moist chamber for twenty-four hours. They were then removed to a greenhouse at a temperature

of about 26° C. Five weeks later when the plants were about eighteen inches high, they had developed six additional leaves (the youngest was not fully unrolled) and secondary rust infection was general. At this time the relative abundance of rust on each species of corn was carefully noted according to the scale for estimating rust as used by the Office of Cereal Investigations, United States Department of Agriculture, and published by Durrell and Parker (2). The two first leaves that were inoculated were not included in taking the rust reading since they had all been inoculated, using artificial technique and consequently were all rather heavily rusted; in fact, some of them were dried up when rust ratings were taken. Nor was the youngest leaf included in the rust readings since, being only partly unrolled it was not old enough to have developed the rust. Hence only five leaves (third to seventh inclusive) were used in taking the rust percentage readings. Seven series of these inoculations were carried out and the readings similarly taken. The results from these seven series were averaged and the averages are given in table 4.

TABLE 4

*Average percentages of rust on the seven species of corn and their rank as to relative susceptibility*

| Species | Average rust percentages | Rank as to susceptibility |
|---|---|---|
| *Zea. saccharata* (sweet) | 74 | 1 |
| *Z. indurata* (flint) | 70 | 2 |
| *Z. amylacea* (flour) | 65 | 3 |
| *Z. indentata* (dent) | 58 | 4 |
| *Z. ramosa* | 44 | 5 |
| *Z. tunicata* (pod) | 32 | 6 |
| *Z. everta* (pop) | 22 | 7 |

These data are in accord with general field observations particularly in regard to the greater susceptibility of sweet corn to corn rust.

## SUMMARY

The results of the investigations herein reported indicate:

1. That the minimum, optimum, and maximum temperatures for germination of urediniospores of corn rust are 4°, 17°, and 32° C. respectively.

2. That the optimum temperature for infection is about 18° C. The minim um and maximum temperatures were not definitely determined but were found to be somewhat below 8° C. and 32° C. respectively.

3. That the germ tubes enter the stomata of the corn plant either with or without the formation of appressoria.

4. That, urediniospores did not overwinter in the vicinity of Madison, Wisconsin, during the winter of 1919–1920.

5. That, while there is no evidence of specialization on the part of the rust, there seems to be rather definite differences in susceptibility to the rust in the different species of corn.

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## BRIEFER ARTICLES

### ADDITIONAL HOSTS FOR BACTERIUM SOLANACEARUM

FREDERICK A. WOLF

One of the striking features exhibited by *Bacterium solanacearum* is its ability to attack a considerable number of species belonging to widely separated families. A total of about 50 species of host plants classified among 9 families have thus far been reported to be subject to attack. Attention was directed to this fact in a previous paper[1] from this laboratory wherein 13 species of plants hitherto not regarded as hosts exhibited well defined wilting or serious injury following inoculation with pure culture. This list has subsequently been enlarged to include castor bean (*Ricinus communis*)[2] and bean (*Phaseolus vulgaris*)[3] both of which have been noted to be naturally infected in Florida. These last reports futhermore include the results of successful artificial inoculations, under certain conditions, into cotton, vanilla, fuchsia, sunflower, garden pea, cowpea, and soybean.

During August 1921, my attention was directed to a wilting of soybeans (*Soja max*) dahlia (*Dahlia rosea*) and cosmos (*Cosmos bipinnatus*). The specimens of soybeans were sent from Columbus, N. C., by Mr. J. R. Sams and those of the other hosts were collected in a garden in Raleigh, N. C. In the case of soybeans, probably because of the woody nature of their stems and petioles, wilting is not a prominent symptom. Affected plants are dwarfed, however, and the foliage becomes prematurely dry and dead. In the case of both dahlia and cosmos, the tops of the plants show a sudden wilting and drooping. During the night, such plants may recover temporarily but after several days, the leaves shrivel, become dry, and remain attached to the plant, which ultimately dies.

No very marked discoloration of the vascular system, except near the base of the stem, was apparent in the case of any of the species. Upon microscopic examination of the xylem tissues, however, bacteria were found to be abundantly present well above the surface of the ground.

[1] Stanford, E. E. and Wolf, F. A. Studies on Bacterium solanacearum, Phytopath. **7**: 155–165; *fig. 1*, 1917.

[2] Smith, Erwin F. Bacterial wilt of castor bean (*Ricinus communis* L.). Jour. Agr. Res. **21**: 255-261, *pls. 55-67*. 1921.

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These bacteria were isolated from each host and invariably in pure culture by the following procedure. The surface of the stems was first sterilized in mercuric chloride. The stems were then cut off with a sterile scalpel and by means of a forceps the plant juice together with the bacteria was squeezed out at the cut end. A platinum loopful of this exudate was spread with a zigzag stroke over the surface of a hardened agar plate. After two days incubation, numerous colonies had developed on all plates. These colonies were well isolated especially near the end of the stroke. Because of their distinct opalescent blue color by oblique light and brownish color by transmitted light, these colonies gave indications that the causal organism was *Bacterium solanacearum*. They were accordingly compared with cultures of this organism isolated from wilted tobacco collected several weeks earlier near Washington, N. C., and with a strain recently isolated from wilted tomatoes from Raleigh, N. C. All were found to be similar in their ability to brown agar media and broths containing peptone. Further, no differences were apparent in fermentation studies with either dextrose, saccharose, lactose, maltose, glycerine, galactose, dextrine and mannite in bouillon consisting of 1 per cent Armour's peptone 0.3 per cent Liebig's beef extract and 0.5 per cent sodium chlorid. Cultures from strains became progressively more alkaline from an initial reaction of pH = 7.2 with each of these carbon compounds, and none formed gas. No inoculations experiments were attempted.

In view of the fact that *Bact. solanacearum* has been repeatedly isolated in this laboratory and has been studied for a term of years, and that the strains from soybean, dahlia and cosmos are like those from wilted tobacco and tomatoes in the above mentioned cultural characters, which are identical with those of *Bact. solanacearum*, the diseased condition of soybean, dahlia and cosmos must have been brought about by this bacterial organism.

## A LEAFSPOT DISEASE OF TOBACCO CAUSED BY PHYLLOSTICTA NICOTIANA E. AND E.

FREDERICK A. WOLF

In the course of studies of diseases affecting the foliage of tobacco, one of minor importance has been noted which apparently has not previously been made the subject of investigation. This disease has been observed for several seasons both upon seedlings in the plant bed and upon more mature plants in the field. It manifests itself by the formation of brownish zonate spots, irregular in outline, which vary in size from 1 to 10 mm. The spots are of the lightest shade of brown near the center and of the darkest zone near the margin. A border of pale green or yellowish green tissues surrounds the invaded dead tissues. Pycnidia are sparsely present in these dead tissues. They can be seen

with difficulty with low magnification because their color is so nearly that of the surrounding tissues. These pycnidia are thin walled, ostiolate and vary in diameter from 75 to 150μ. The conidia are hyaline, an occasional one is one-septate, and they measure 6–10 × 3–3.5μ.

Isolations from single conidia were effected by the dilution poured plate method. The course of germination is not different from many forms which have been described. There is at first a very considerable swelling followed, within 18 hours, by the formation of one or two terminal germ tubes. In the course of a few days, white, floccose colonies are formed. The portion of the mycelium in contact with the substratum blackens with age. In such media as 1 per cent dextrose plus 2 per cent agar, pycnidia are sparsely developed.

Seedling tobacco plants in flats in the greenhouse were atomized with a suspension of conidia from pure culture in tests to determine pathogenicity. Inoculations were made late in the afternoon and the flats were covered during the night with a sheet of paper in order to preserve a high relative humidity. Infections were evident within a week and within 7 to 10 days later, mature pycnidia were present within the invaded tissues. These were similar morphologically to those from which the original isolations were made.

It has been impossible to establish with certainty, the identity of this organism by comparison with published descriptions of the several species of Phyllosticta occurring on *Nicotiana tabacum*. It differs from *Phyllosticta tabaci* Pass., collected in Italy, principally in the appearance of the lesions and color and distribution of the pycnidia. It differs from *P. capsicola* Sacc. and Speg., also described from material collected in Italy, since the pycnidia of this species are grouped, the ostiole is somewhat beaked, and the conidia are curved and guttulate. *P. nicotianicola* Speg. on *Nicotiana acutiflora* from Argentine, differs in that it forms whitish leafspots, and its conidia are sub-cylindrical although its pycnidia with respect to distribution, size, shape and color are like those of the organism in question. It is manifestly very different from *Phoma nicotianae* Maubl. from France, and it is not believed that sufficient significance should be attached to the occasional occurrence of septate conidia to warrant regarding it as an Ascochyta.

Apparently the only known North American Phyllosticta on tobacco is *P. nicotiana* E. and E., from North Carolina, type specimens of which are missing from the Ellis and Everhart exciccati in the herbarium of the New York Botanical Garden.[1] The meagreness of the original description[2] of this form gives little with which to make comparison

[1] Thanks are due to Dr. F. J. Seaver for his courtesy in examining the collections of the N. Y. Botanical Garden for me.

[2] Ellis, J. B., and Everhart, B. M. New species of North American fungi, Proc. Acad. Nat. Sci. Phila., p. 157, 1893.

with the material in hand. Whether or not they are identical can only be a matter of opinion. The discrepancy in the size of conidia, since those from Ellis and Everhart's type are described as about half that of my material, may be accounted for by an error in calibration or a shrinkage of conidia through desiccation before measurements were made. Since this cannot now be determined because of the loss of type material and since it does not appear that confusion would arise were one to reestablish the type on the basis of the material in hand, it is regarded as identical with *Phyllosticta nicotiana*. In order to preserve the writer's material, therefore, and make it available to students of tobacco diseases, it has been deposited in the herbarium of the Office of Pathological Collections of the Bureau of Plant Industry, Washington, D. C.

## BANANA FRECKLE IN THE PHILIPPINE ISLANDS

H. ATHERTON LEE

WITH ONE FIGURE IN THE TEXT

A black spotting is very common upon the banana fruits in the public markets in the Philippines. Both green and mature fruits show this disease. The spots are a dark reddish brown on certain green varieties but on others are black, minute, erumpent, and hard. Usually they occur in great numbers and in some cases in masses on the fruits. The spots upon the leaves are similar to those on the fruits but frequently run in streaks giving much the same appearance as melanose or tearstain on oranges. An affected leaf rubbed with the finger has a rough feeling suggestive of sandpaper. The Latundan, Borongan, Lacatan, and Saba (local dialect) varieties of *Musa sapientum* are affected.

Examination of the black spots shows the presence of pycnidia, which contain densely granular, oval, or irregularly shaped spores, having a thick hyaline envelope, and frequently a short hyaline appendage. The pycnidia and spores resemble very closely those of the fungus described as *Phoma musae*, Carpenter. Attempts to culture the organism have been unsuccessful, this experience being similar to that described by Carpenter[1] who mentions in his description that he was unable to grow it. The black spotting is entirely similar to the disease banana freckle as pointed out to the writer in the Hawaiian Islands by Professor Carpenter.

The disease is much more abundant at the close of the wet season than during the dry season. Since bananas in the Philippines are grown entirely for local consumption, the disease here does not cause the loss which occurs to the banana industry in the Hawaiian Islands.

[1] Carpenter, C. W. Banana freckle or black spot disease. Report of the Hawaii (Federal) Agric. Station, **1918**: 36–40. *Pl. 8–9*. 1919.

A recent trip into the Sulu Archipelago and the Island of Mindanao has shown this disease to be widespread throughout those regions. In as much as these islands are but sparsely populated and importations of bananas from the western hemisphere have been unknown, the indications are that banana freckle is indigenous or at least of long duration here. In this connection it is of interest to quote from Professor Carpenter's report.

". . . . . A year ago this disease appeared to be confined almost exclusively to the valley of Kalihi, (Territory of Hawaii) where it was serious but it could scarcely be found in Manoa Valley, 5 miles distant. It is now present to an alarming extent in the plantations of Moanalua, Pearl City, Mokuleia and Kahaluu, as well as in Kalihi and Manoa. During a recent trip to Kalihi Valley, it was impossible to find a bunch of fruit not affected . . . . . ."

"Whether this is a new disease in Hawaii cannot be decided, though the writer inclines to the view."

Fig. 1. Freckle disease of bananas, similar to that described from Hawaii by Carpenter. On Latundan variety, from Manila markets.

The finding of banana freckle, so commonly and so widely distributed in the Philippines, would apparently corroborate Carpenter's view. There is a continual migration of labor from the Philippines to the sugar plantations of Hawaii and in view of this it is easy to understand the course by which a few affected fruits could reach the banana plantations of Kalihi Valley, adjacent to Honolulu.

BUREAU OF SCIENCE,
MANILA, P. I.

## *ABSTRACTS OF PAPERS PRESENTED AT THE FIFTH ANNUAL MEETING OF THE PACIFIC DIVISION, AMERICAN PHYTOPATHOLOGICAL SOCIETY, BERKELEY, CALIFORNIA, AUGUST 4 to 6, 1921.*

*Experiments on the use of oiled fruit wraps for the control of apple scald.* CHARLES BROOKS, J. S. COOLEY, AND D. F. FISHER.

Experiments conducted during the past year have confirmed results previously reported by the writers that scald can be successfully controlled under the most unfavorable storage conditions by wrapping apples in paper infiltrated with various oils which absorb the injurious respiration gases of the fruit. Oils heretofore used have tainted the fruit but during the past season an odorless and tasteless type of oil has been tested and has proved completely successful.

It was also found that apple scald could be arrested or reduced after several months storage by removing the common paper wraps and substituting oiled paper; likewise that if the fruit was first stored in oiled paper scald did not result when it was repacked in common paper after as short a time as one month.

*An outbreak of powdery mildew (Podosphaera leucotricha) on pears.* D. F. FISHER.

An outbreak of powdery mildew on pears occurred in the Central Washington fruit districts in the spring of 1921. Twig and foliage infection was not general but was confined to tender "water-sprouts" and terminals. The fruit, however, was very generally infected, orchards of D'Anjou variety commonly showing nearly every pear infected. Louise Bonne was nearly as susceptible as D'Anjou but Bartlett was considerably less so, while Flemish Beauty and Winter Nelis were markedly resistant. Conidia developed very freely and about July 1 perithecia began to appear as *Podosphaera leucotricha*, the same as on the apple. The disease has been epidemic on apples in this region since 1914 but has heretofore been noted only rarely on pears, although the trees are generally interplanted. The morphological characters of the fungus on pears do not differ from those found on apples, but the general occurrence of perithecia on the fruit instead of twigs is a noteworthy difference. The effect of the disease upon the fruit is the production of a black or russetted disfiguration, and in some cases a distortion of shape somewhat like early scab infection. There is also evidence that the disease caused an abnormal drop of fruit especially on D'Anjou.

*Notes on bacterial gummosis of stone fruits.* J. T. BARRETT.

The disease designated "bacterial gummosis" of stone fruits and caused by *Pseudomonas Cerasus* Griffin, has been under observation in California since 1916. Recognized at that time in only two rather widely separated counties it is now known to be widely distributed throughout the State. Attention was first directed to severe attacks on the apricot which in California seems to be very susceptible to the disease. There is, however, a great difference in severity on the apricot in different sections, and some evidence that there exists a difference in varietal susceptibility. In the northern part of the State the cherry is also attacked but not so severely as has been reported in the more humid sections of Oregon. In April of this year young peach buds in the nursery were attacked, many being killed, others badly injured. A difference in varietal resistance was apparent. In the same nursery and others, one very distant, young apricot

buds were similarly but more severely attacked. In one case 75 per cent of the new buds were killed in a three acre tract. Infection may take place from October to May but greatest activity on the apricot has been observed from January to April. In some of the old cankers the organism lives over the summer and together with spring infected fruit spurs furnish a scource of inoculum for the following season. Preliminary tests indicate that from 20° C. to 27° C. is the range of temperature for optimum growth of the organism.

*Die-back of loganberry in the Northwest.* S. M. ZELLER.

Die-back of the canes of loganberry is very prevalent in the Northwest this year, especially where the canes were left down during winter. In yards having the canes trained up in the fall no die-back is manifest. *Mycosphaerella rubina* has been identified with some of the die-back but usually as an affectation subsequent to some other causal condition. Low temperatures sufficient to injure canes have not been experienced and the root systems are usually in normal vigor. Evidence points to the conclusion that the canes are devitalized by the extreme moist conditions to which they are subjected during the winter rather than to the effects of any parasitic organism or low temperatures.

*Effect of alkaline sprays on the size of sweet cherries.* D. F. FISHER.

During spraying experiments for the control of brown rot of sweet cherries at Salem, Oregon, in 1915, 1916, and 1917, it was observed that a reduction in size of the fruit resulted from the application of fungicides. The repeated heavy application of self-boiled lime-sulphur 8–8–50 and Bordeaux 2–4–50, renders the fruit unmarketable through shrivelling, and the trees were not picked. The dwarfing was not as noticeable with commercial lime-sulphur 1–50. An explanation of this dwarfing was sought in special experiments in which various fungicides and components of the same were applied to different varieties both at Salem, Oregon, and Wenatchee, Washington. Check or unsprayed areas were retained on each tree to give comparable fruit and to eliminate variations due to individuality of the trees. Two applications were made, one as soon as the cherries began to color and another about two weeks later. In each case reduction in size of the ripe cherries was in proportion to the amount of alkaline material in the spray. A lime and lampblack wash resulted in no greater dwarfing than occurred from the application of lime alone, indicating that reduction of illumination was not the cause of the smaller fruit. The results indicate that dwarfing by alkaline sprays is brought about through excessive transpiration or water loss occasioned by the destruction of the waxy bloom.

*The parasitism of bastard toad flax (Comandra pallida A. DC.).* D. F. FISHER.

A study has been made of the parasitism of bastard toad flax in the region about Wenatchee, Washington, where this plant, *Comandra pallida*, is commonly found among the native vegetation of sagebrush lands. It was first observed growing under apple trees in a neglected orchard in 1919, but its parasitism on apple roots was not established until a year later when a more detailed survey and study was made. At that time specimens were sent to Dr. C. V. Piper and were identified by him as *Comandra pallida*. This parasitic attack on apple roots was described by Piper before the Botanical Society of Washington. *Comandra* has since been found parasitic on roots of the peach but this host was less affected than the apple. Pear trees and alfalfa growing in the same orchard were not attacked. Among the native plants the following were attacked; sagebrush (*Artemesia tridentata*), lupine (*Lupinus suksdorfii*), and yarrow (*Achillea millefolium*).

*European canker in Pacific Coast States.* S. M. ZELLER AND C. E. OWENS.

The European canker of apple (caused by *Nectria galligena* Bres.) has been known in Oregon since 1918, but not until after the extreme low temperatures experienced in December, 1919, was its wide distribution noted. At present the hosts for this canker, upon which the Nectria or Fusarium stages have been definitely identified in Oregon are several varieties of apples, namely; Red Cheek Pippin, Bismark, Delicious, Bellflower, Ortley, Spitzenburg and Newtown; D'Anjou, Howell, and Bosc pears; *Quercus garyana, Acer macrophyllum, A. circinatum, Cornus Nuttallii* and *Salix* sp. It is reported from Humboldt Company, California, on apple. It is extremely virulent on Bosc and D'Anjou pears, producing cankers 20–22 inches in length in one season.

*Occurrence of Tylenchus dipsaci on alfalfa in Oregon.* M. B. MCKAY.

Specimens of diseased alfalfa plants sent to our laboratory from one farm under irrigation near Hermiston, Oregon, in June, 1921, showed the presence of and typical injury from the leaf and stem-infesting nematode, *Tylenchus dipsaci* (Kühn) Bastian. This pest has apparently not been previously reported on alfalfa in America though it has long been known on this crop in Europe and southern Africa where it has caused serious losses. The infested stems sent were usually much shortened and noticeably swollen and the epidermis over the infected areas was considerably wrinkled and corrugated. Some of the stems were killed entirely. As a consequence the stand was appreciably thinned and the first cutting of hay was light. It is not known how long the pest has been present on alfalfa in the region though similar injury was noted by the grower last year. The nematode from alfalfa was readily transferred by inoculations to clover on which it is causing typical injury. This nematode occurs as an economic pest also on clover and strawberry in Oregon and has been found causing damage in both irrigated and non-irrigated regions.

*Minimum incubation periods of causative agent of curly leaf in beet leafhopper (Eutettix tenella Baker) and sugar beet.* HENRY H. P. SEVERIN.

The beet leafhopper is non-virulent when it hatches from the egg. Curly leaf is not transmitted through seeds from "stechlinge" affected with the disease before and after transplanting. The beet leafhopper is not a mechanical carrier of curly leaf, nor a mechanical carrier in mass infection of a beet. The minimum incubation period of the causative agent of curly leaf in the beet leafhopper required four hours at the following temperatures: maximum 103°, minimum 94°; and mean 100° F.; and five days in the sugar beet at the following temperatures: maximum 93.6°; minimum 53.3° and mean 73.5° F.

*Transit diseases: An important factor in the cost of fresh vegetables.* GEO. K. K. LINK.

*Curly top of beet. Is it a mosaic disease?* EUBANKS CARSNER.

*A Phomopsis from the Isle of Pines.* W. T. HORNE.

Grape fruit from the Isle of Pines, W. I., were secured from Mr. Frederick Maskew, State Commissioner of Horticulture, Quarantine Division, San Francisco, in October 1917, with stem end rot. From one of these fruits a fungus of the general Phomopsis character was isolated, which was capable of producing typical stem end rot in oranges and grape fruit and developed readily in ordinary culture media. In green citrus twigs in culture tubes it developed readily and formed pycnidia copiously. The fungus does not correspond exactly in culture characters with *Phomopsis citri*, Fawcett, from Florida, being apparently more vigorous.

*Some notes on two cars of grapefruit from the Isle of Pines.* W. T. HORNE.

Two cars of grape fruit were received in San Francisco in October 1917. A number of these fruits were examined and the more common blemishes were noted and their significance for the California citrus industry discussed. These included rust mite injury, tear stain, melanose (?), scab, black thrips rings, storage spot, gum spot, Diplodia stem end rot, Phomopsis stem end rot, *Myriangium sp.*, *Sphaerostilbe cocophila* Tulasne.

*Pathogenicity of the olive knot organism on hosts related to the olive.* C. O. SMITH.

This organism, *Pseudomonas savastanoi*, has been studied by other investigators and artificial inoculation on hosts other than the olive were always either doubtful or negative. The inoculations of hosts closely related to the olive was made during 1919 to 1921. The following hosts when inoculated gave knots or galls that closely resembled those on the olive: *Fraxinus velutina*, *Fraxinus floribunda*, *Adelia acuminata*. Other hosts reacted differently and definite lesions 5 to 15 mm. in size, having hypertrophies at the margin of wound, were developed. At the margin small galls 2 to 3 mm. in diameter often formed. Hosts reacting as described are *Chionanthus virginica*, *Osmanthus aquifolia*. Hosts having small, point-like growths which on further experimentation would probably give positive results, are privet, *Ligustrum ovalifolium* and *Jasminum primulinum*. Negative results on *Osmanthus fragrans*, *Vinca theretia nerifolia*, *Nerium oleander*, *Carissa grandiflora*, *Chrysanthemum frutescens*, *Elaeagnus augustifolia*, lilac, various species of prunus, and *Coprosma baucrii*. Positive results only obtained on hosts closely related botanically to the olive.

*Some studies relating to infection of and resistance to walnut blight, Pseudomonas juglandis.* C. O. SMITH.

The leaf and catkin buds situated near blight lesions may have the blight organism on their surface before new growth starts, as shown by dilution plates. Artificial inoculation on dormant buds by puncture, brushing, or atomizing, develops little or no infection on new growth. Buds advanced so as to show green leaf tissue may be readily inoculated. Catkins themselves become infected, showing black, watery areas. Pollen from such catkins when shaken into petri dishes with agar develop blight colonies. Soil has been tested as a carrier by inoculating and testing same by poured plate method, and by direct inoculation of nuts. Organism could not be cultured, or cause infection, after about nine days. Commercial varieties of English walnut artifically inoculated to test resistance gave Ehrhardt 27, Eureka 29, Placentia 34, and Seedlings 49 per cent.

*Internal decline of lemons.* E. T. BARTHOLOMEW.

This is not a report of completed work but merely a description of the disease. It is a physiological malady. It is often spoken of commercially as "Blossom end decay," "Tip deterioration," "Pink tip," etc. It is characterized by the breaking down and drying out of the internal tissues usually at the blossom end of the fruit. It is usually first noticeable in the inner layers of the white parenchymatous tissue of the rind. The cells become disintegrated and a brown to pink gummy substance takes their place. From here the disease rather rapidly progresses into the pulpy tissues below and they become dry and light brown in color. The trouble does not appear until the fruit is about ready to pick, but then it may be found in green, silver, or yellow fruits, usually in the two latter. The disease does not appear to progress in the fruits after they have been taken from the trees. This disease is of very great economic importance for it often makes from 40 to 50 per cent of a pick of no value except for the by-products laboratory.

*Acid and water content of lemon fruits at different stages of development.* E. T. BARTHOLOMEW.

To study the cause of "Internal Decline," young lemons were measured, tagged, and remeasured once every month until mature. Each month some fruits were brought to the laboratory and tests were made to determine relative percentages of acidity and water content in the ends of the fruits. The acidity was found to be approximately the same in both ends of the lemons at all stages. The acidity gradually increases from about pH 4.46 in fruits ¾ inch in diameter to about pH 2.23 in mature fruits. Fruits remaining on the tree after maturity show a small decrease in acidity. The acidity of the abnormal tissue was found to be pH 0.1 less than that of normal tissue. The water content of the blossom end is usually slightly greater than that of the stem end. Both rind and pulp were included in determining the water content. The water content of lemons ¾ inch in diameter is about 53 to 55 per cent; that of mature fruits about 88–90 per cent. Fruits set in the spring grow much more rapidly and mature much quicker than those set at any other time. It may take 7 to 12 or even 14 months for lemons to mature, depending upon the time of the year at which they set.

*A Phomopsis of Citrus in California.* H. S. FAWCETT.

A Phomopsis somewhat resembling *P. citri* in Florida, and first found by O. F. Burger in California lemon shipments to Philadelphia, has been studied. Up to the present time it has been found only in Santa Barbara and Los Angeles counties. This California Phomopsis in cultures has a somewhat different mycelial growth, sporulates more freely and has different temperature relations to that of the Ebrida Phomopsis. Its ability to enter and break down fruit has been shown by inoculation experiments to be very weak as compared to the Florida Phomopsis. It has been found in the packing house only on old overripe fruit, and in the orchards on a few dead twigs only after considerable search. Traces of what appeared to be mild melanose markings (a conspicuous result of *Phomopsis citri* attack in Florida) were also found on a few specimens of pomelo fruits in Santa Barbara County. The lack of virulence of this Phomopsis and, therefore, the minor importance of the effect produced by it probably accounts for its having been previously overlooked.

*The relation of Citrus blast to certain environmental factors.* H. S. FAWCETT.

The organism (*Bacterium citriputeale*), which has been shown to be responsible for both citrus blast and black pit is markedly sensitive to slight changes in climatic conditions. It is active only in the rainy season during a short period in late winter and early spring. The history of its occurrence since 1912 at Oroville studied in connection with weather data, shows that whenever the number of rainy or cloudy days falls below normal the disease is mild or unimportant and whenever the rainy or cloudy days are above normal the disease is severe. It has also been shown statistically that the disease has a direct relation to injuries to the foliage by winds when accompanied or followed by rains. The organism has a low optimum temprature for growth and infection and is dependent upon cool as well as moist weather condition and injuries for infection and development.

*Some fig diseases.* EDITH H. PHILLIPS.

1. Fig smut. An Aspergillus attacks the insides of the fruits as they begin to dry. This fungus has been found to carry over in small twigs.
2. *Sclerotinia libertiana* causes a die-back of fig twigs in the spring.
3. A Botrytis also causes a die-back. It enters the twigs through hang-on figs in the fall, or through frost-injured tissue in the spring.
4. An undetermined fungus causes a rot of fig fruits and subsequent cankers at the points where the rotted figs hang.

*Data relative to the germination of aeciospores, urediniospores and teliospores of Puccinia coronata Cda.* G. R. HOERNER.

Summary of results: Aeciospores from herbarium specimens of Rhamnus were not viable after a period of 167 days from date of collection. Urediniospores from herbarium specimens of *Avena sativa* L. proved to be viable as long as 87 days after date of collection. Unprotected, urediniospores lost their viability within 22 days with a minimum temperature during this period of —27° F. and a maximum of 42° F. When afforded protection with a temperature range similar to the unprotected, these spores remained viable as long as 44 days. Exposed to light, viability of urediniospores was lost within 23 days, during which period the maximum temperature was 86° F. and the minimum 29° F. Kept in the dark, urediniospores at similar temperatures to those exposed to light, remained viable as long as 79 days. Urediniospores germinated at a temperature as low as 7° C. with an optimum of 18° C. and a maximum of 32° C. Teliospores developed on oat seedlings in the greenhouse and not afforded a period of overwintering, did not germinate. Previous to overwintering and as late in the spring as May 2, teliospores developed in the field were incapable of germination.

*Miscellaneous studies with crown rust of oats.* G. R. HOERNER.

Summary of results: Urediniospores borne on the surface of oats seed do not offer a ready means of infecting seedlings developed from these seeds. Seedlings of oats emerging through soil heavily covered with viable urediniospores are not readily infected. Under Minnesota conditions, a perennial mycelium capable of producing a new crop of urediniospores after overwintering, does not exist. What the situation is in the case of wild grasses has not been determined. Urediniospores do not remain viable over winter on oats, nor does continued production take place. What the situation is in regard to wild grasses has not been determined. Environmental factors influenced the development of the rust on oats as well as the rate of pustule formation. Etiolation brings about the early formation of telia on oat seedlings. Anthocyanin pigment formation surrounding uredinia on infected oat leaves is a common phenomenon though not correlated with resistance or susceptibility. The appearance of telia on seedling oat leaves is not a reliable basis for determining resistance of oat varieties.

*Observations on the nature of resistance to stem rust attack in certain resistant and susceptible wheats.* RUTH F. ALLEN.

*Resistance in oats to stem rust attack.* W. W. MACKIE AND RUTH F. ALLEN.

*Recent investigations on rice disease in the United States.* W. H. TISDALE.

*The present status of cereal pathology in the United States.* H. B. HUMPHREY.

*The use of chemical dusts to prevent bunt.* W. W. MACKIE AND FRED. N. BRIGGS.

*The relation of soil moisture and soil temperature to bunt infection in wheat.* C. W. HUNGERFORD.

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